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(54) **ANTI-PMEL17 ANTIBODIES AND IMMUNOCONJUGATES**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The invention provides anti-PMEL17 antibodies and immunoconjugates and methods of using the same.

67 Claims, 17 Drawing Sheets

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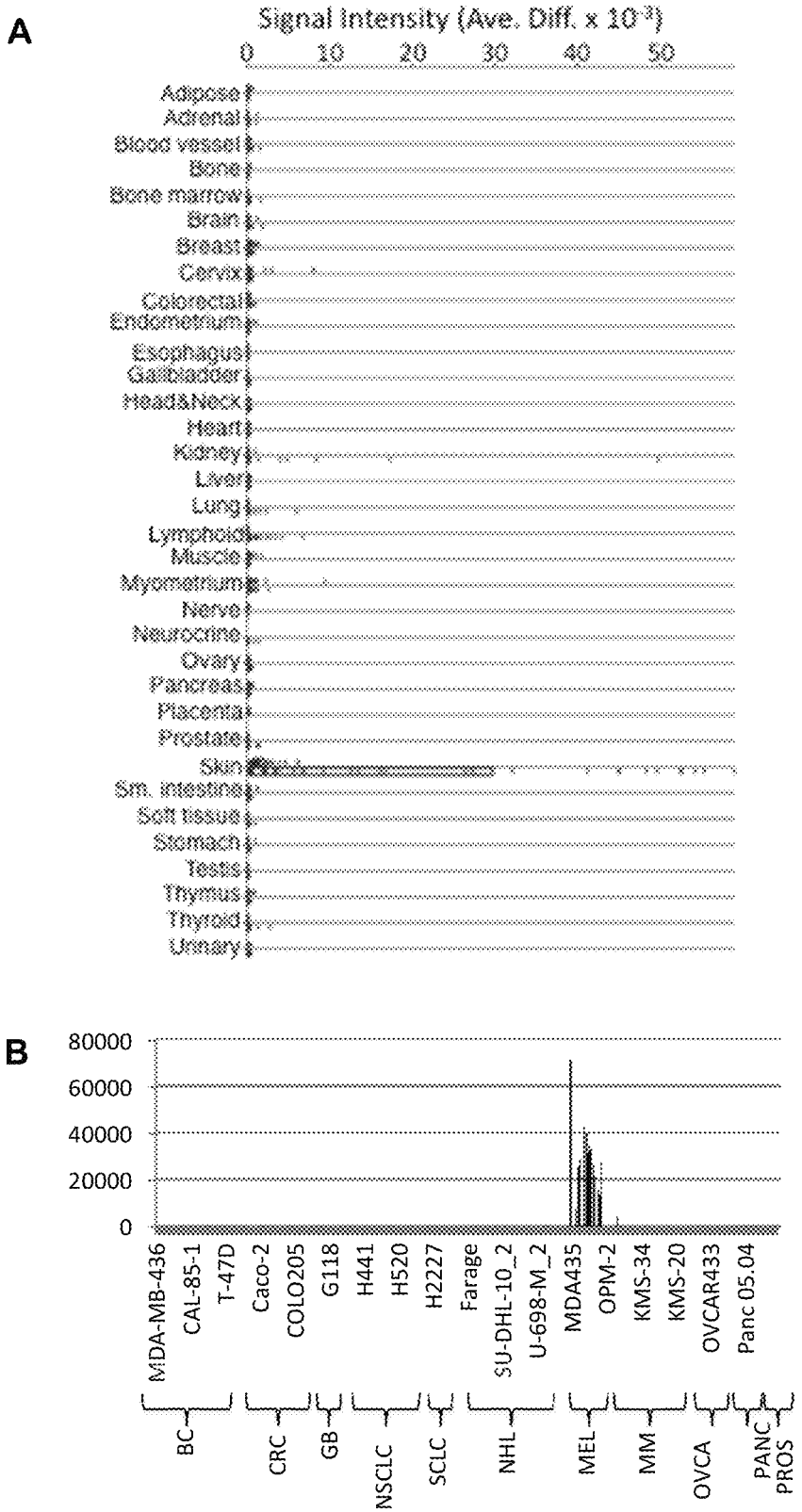
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Signal Intensity (Ave. Diff. x 10⁻³)
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10
20
30
40
50
Adipose
Adrenal
Blood vessel
Bone
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Brain
Breast
Cervix
Colorectal
Endometrium
Esophagus
Gallbladder
Head&Neck
Heart
Kidney
Liver
Lung
Lymphoid
Muscle
Myometrium
Nerve
Neurocrine
Ovary
Pancreas
Placenta
Prostate
Skin
Sm. intestine
Soft tissue
Stomach
Testis
Thymus
Thyroid
Urinary
B
80000
60000
40000
20000
0
MDA-MB-436
CAL-85-1
T-47D
Caco-2
COLO205
G118
H441
H520
H2227
Farage
SU-DHL-10_2
U-698-M_2
MDA435
OPM-2
KMS-34
KMS-20
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NHL
MEL
MM
OVCA
PANC
PROS

FIG. 1

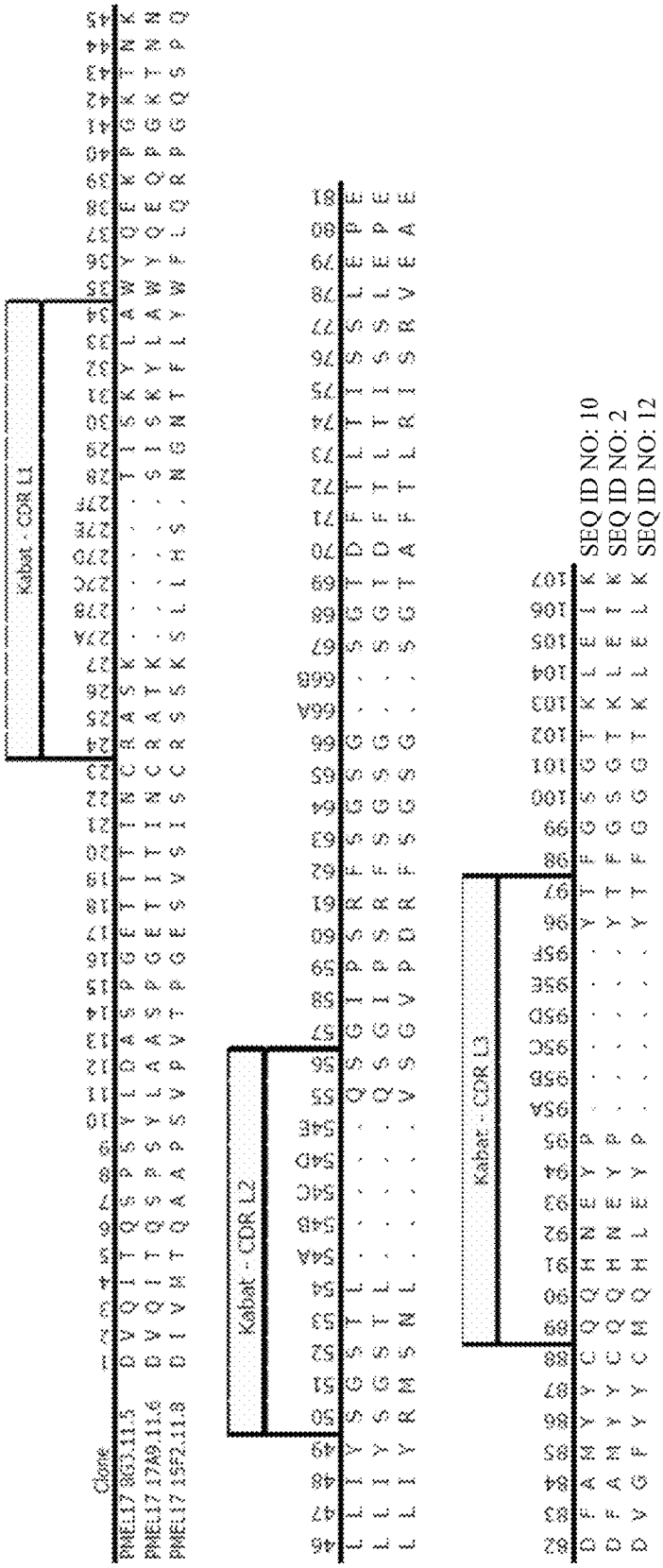
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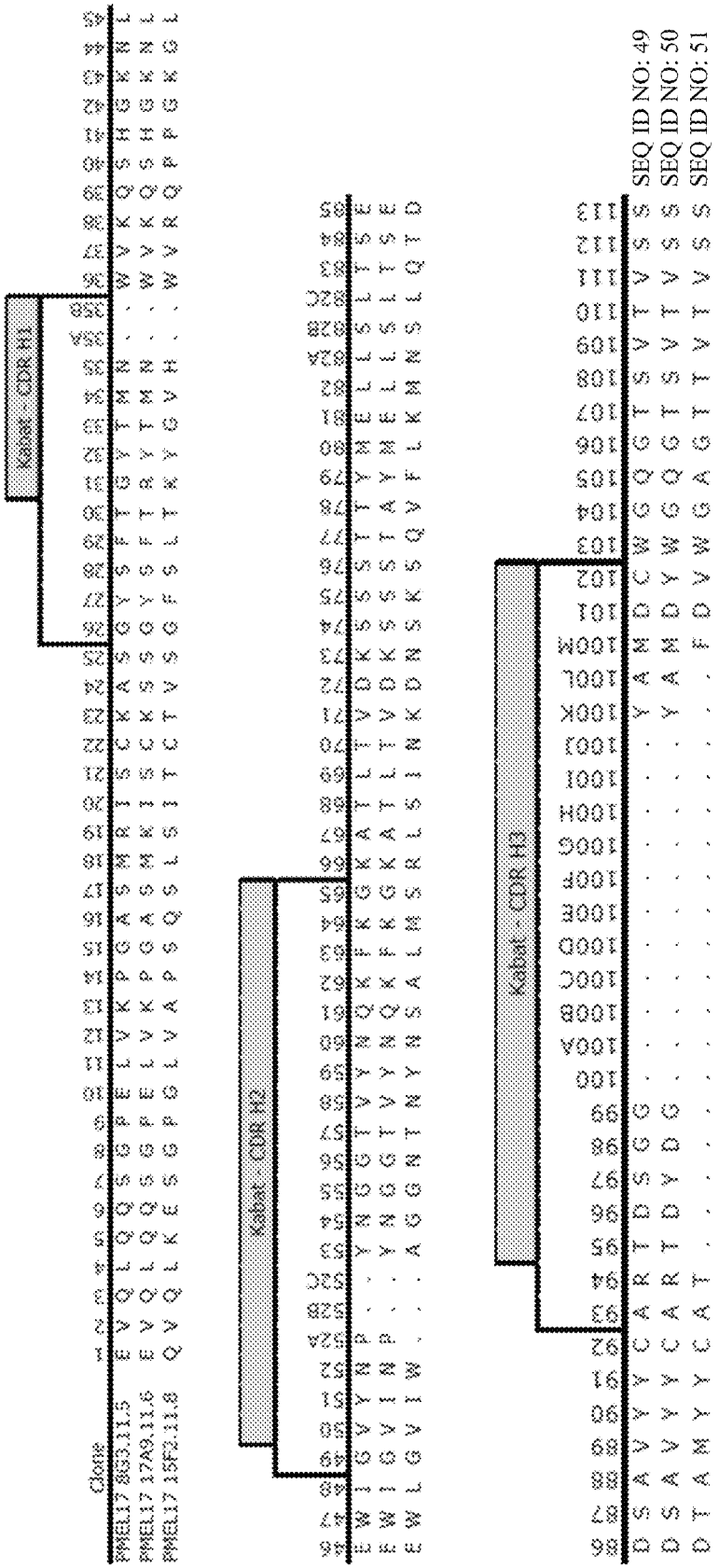
FIG. 2A


Clone
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PMEL17 17A9.11.6
PMEL17 15F2.11.8
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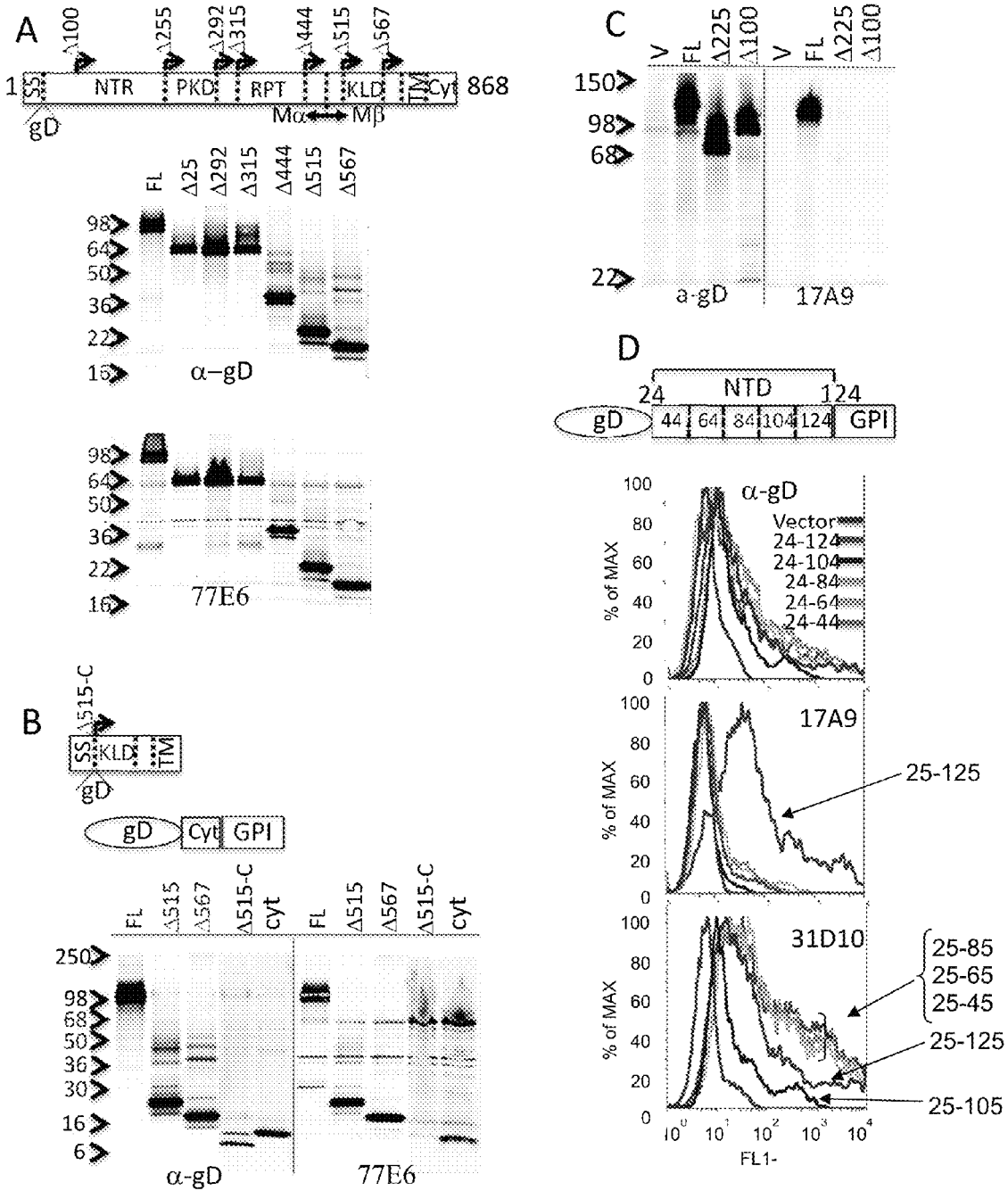
FIG. 2B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Δ100
Δ255
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NTR
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RPT
KLD
TM
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868
gD
Mα
Mβ
FL
Δ25
Δ292
Δ315
Δ444
Δ515
Δ567
98
64
50
36
22
16
α–gD
77E6
B
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SS
KLD
TM
gD
gD
Cyt
GPI
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98
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50
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150
98
68
22
a-gD
17A9
D
24
NTD
124
gD
44
64
84
104
124
GPI
α-gD
Vector
24-124
24-104
24-84
24-64
24-44
% of MAX
17A9
25-125
31D10
25-85
25-65
25-45
25-125
25-105
FL1-

FIG. 3

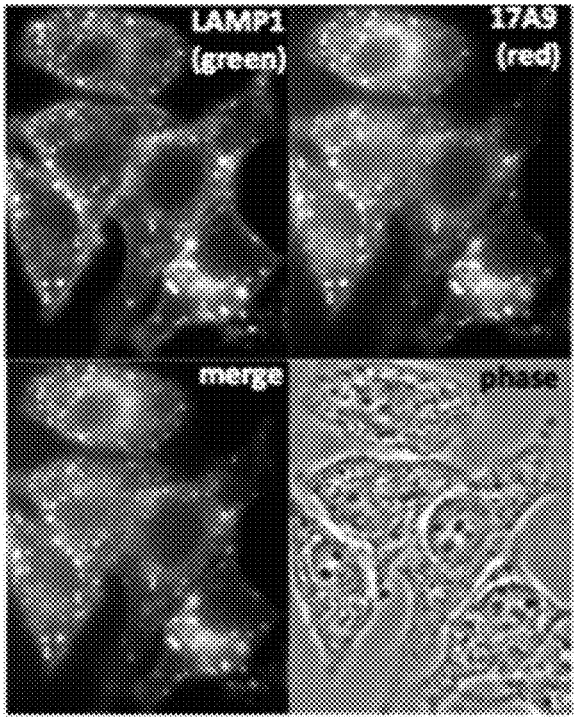

LAMP1
(green)
17A9
(red)
merge
phase

FIG. 4

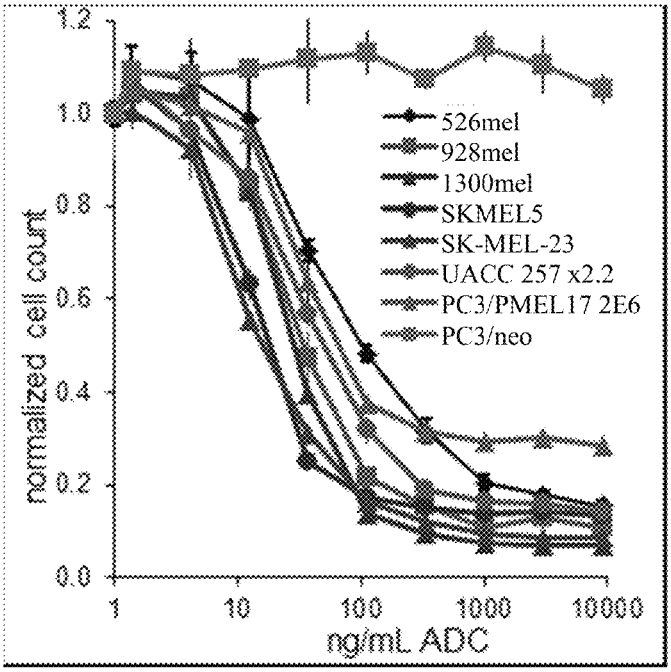

1.2
1.0
0.8
0.6
0.4
0.2
0.0
normalized cell count
1
10
100
1000
10000
ng/mL ADC
526mel
928mel
1300mel
SKMEL5
SK-MEL-23
UACC 257 x2.2
PC3/PMEL17 2E6
PC3/neo

FIG. 5

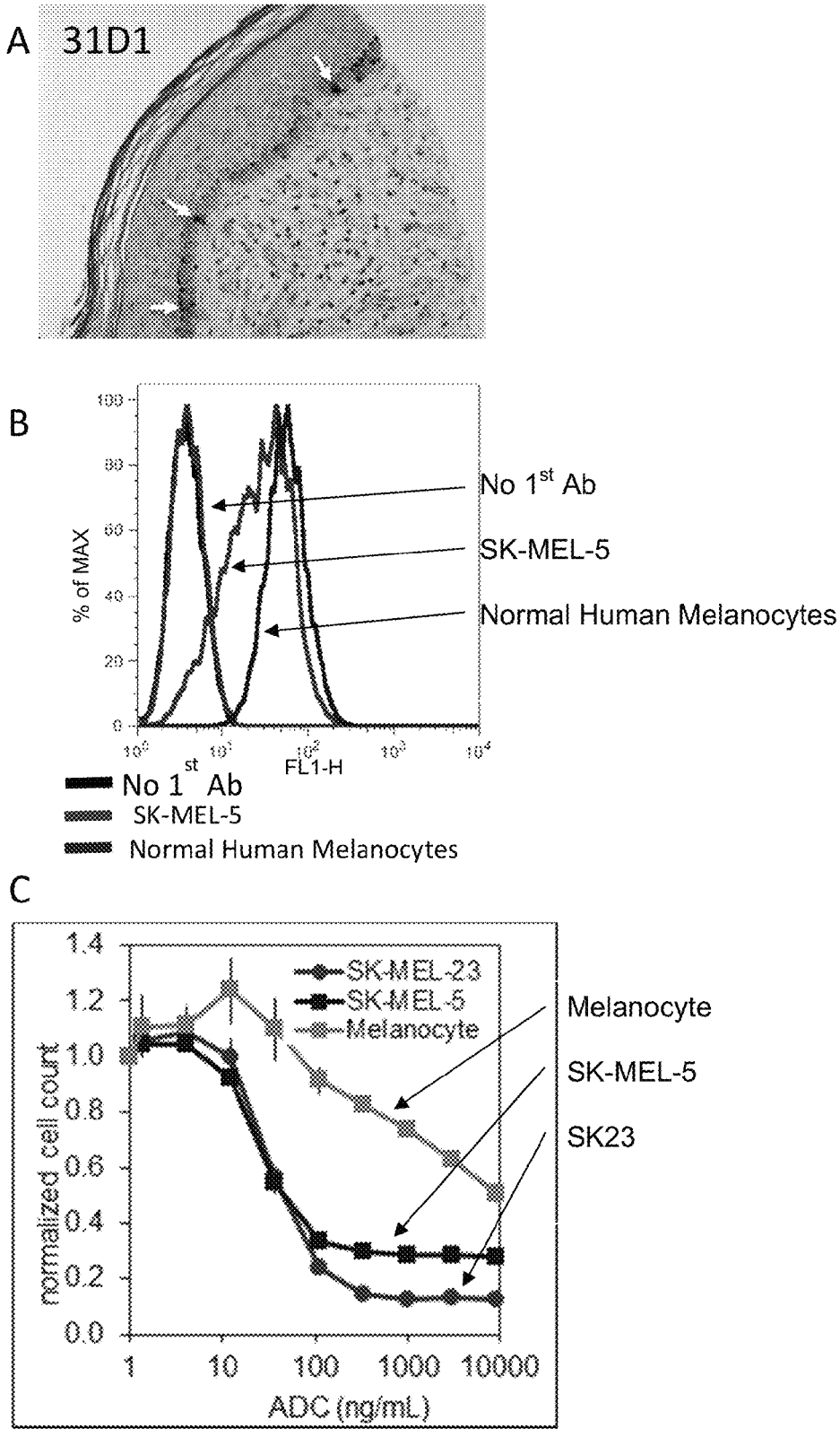
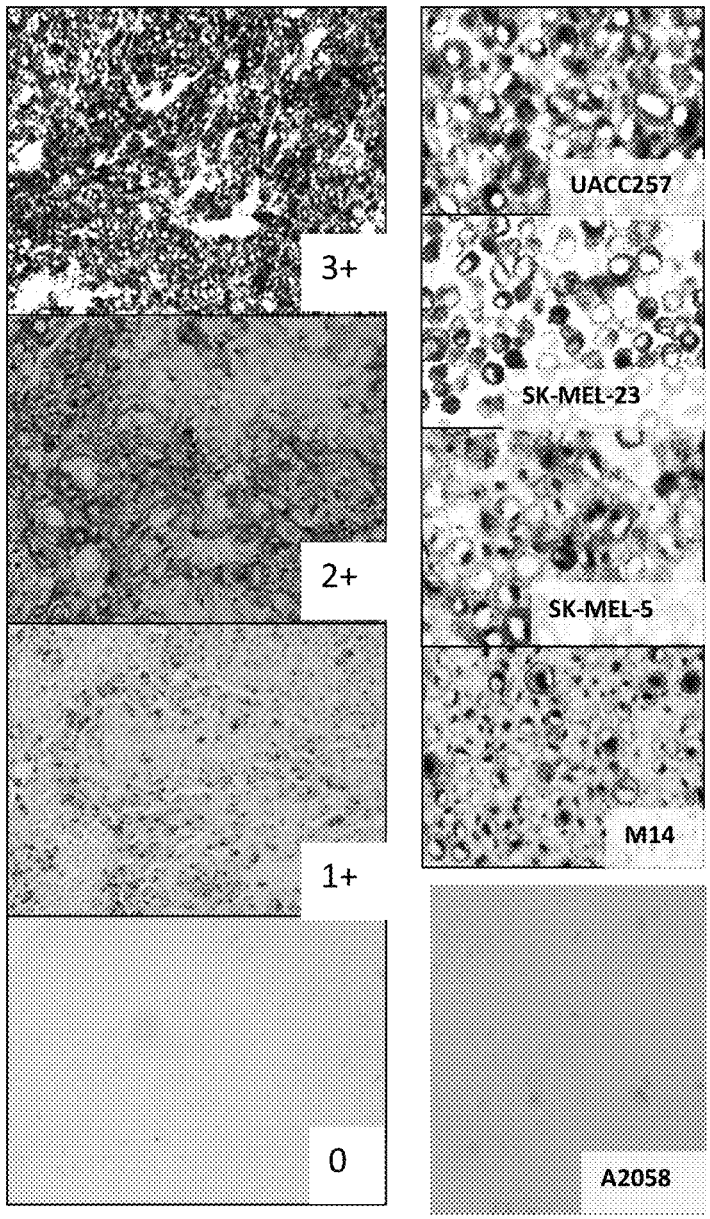

A
31D1
B
% of MAX
FL1-H
No 1st Ab
SK-MEL-5
Normal Human Melanocytes
C
normalized cell count
ADC (ng/mL)
SK-MEL-23
SK-MEL-5
Melanocyte
Melanocyte
SK-MEL-5
SK23

FIG. 6

A



B IHC scores for human melanoma (n=58)

Score	0	1	2	3
31D1	10	15	20	13
17A9	21	17	13	7

FIG. 7

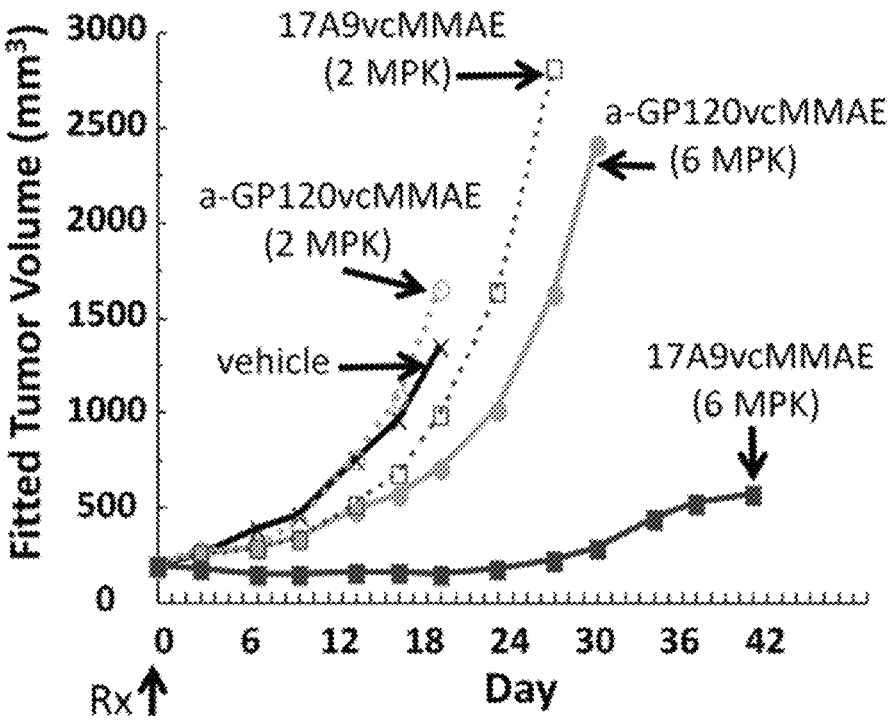

Fitted Tumor Volume (mm³)
3000
2500
2000
1500
1000
500
0
17A9vcMMAE
(2 MPK)
a-GP120vcMMAE
(6 MPK)
a-GP120vcMMAE
(2 MPK)
vehicle
17A9vcMMAE
(6 MPK)
0 6 12 18 24 30 36 42
Rx
Day

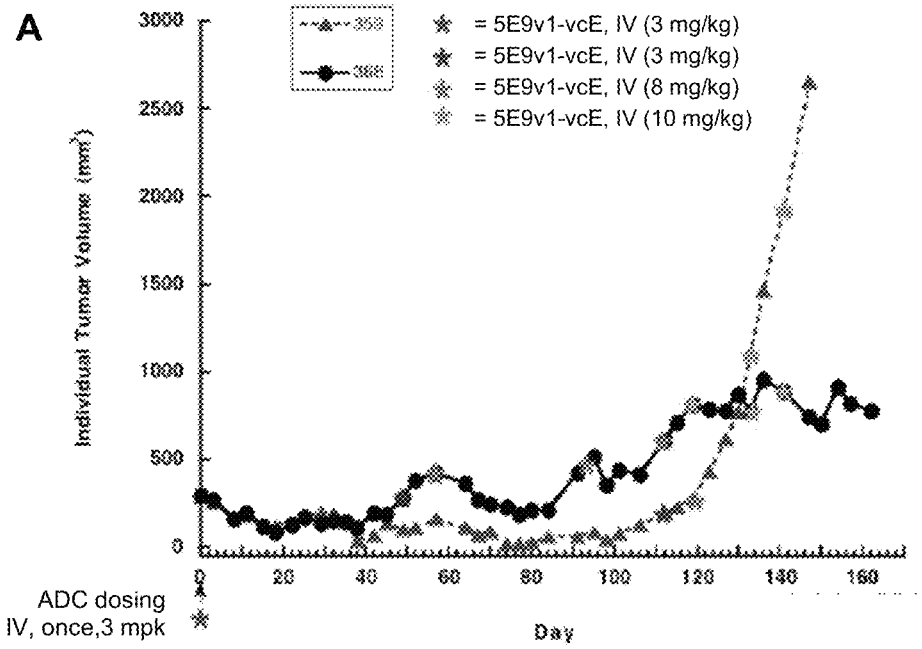
FIG. 8

```
rat     -MGVQRRCFLPVLVLGALLALGSIEGSRNQNHGVSRQLVTKVWNKQLYPEWTEVQGSNC  59
mouse   -MGVQRRSFLPVLVLSALLAVGALEGSRNQDWLGVPRQLVTKTWNRQLYPEWTEVQGSNC  59
human   MDLVLKRCLLHLAVIGALLAVGATKVPRNQDWLGVSRQLRTKAWNRQLYPEWTEAQRLDC  60
cyno    ----------------------------KGPRNQDWLGVSRQLRTKAWNRQLYPEWTEAQRLDC  36
                                     : .***:* **.*** **.**:********.*  :*

rat     WRGGQVSLKVRNDGPTLVGANTSFSIALHFPGSQKVLPDGQVIWVNNTIINGSQVWGGQP 119
mouse   WRGGQVSLRVINDGPTLVGANASFSIALHFPGSQKVLPDGQVIWANNTIINGSQVWGGQF 119
human   WRGGQVSLKVSNDGPTLIGANASFSIALNFPGSQKVLPDGQVIWVNNTIINGSQVWGGQP 120
cyno    WRGGQVSLKVSNDGPTLIGANASFSIALNFPGSQKVLPDGQVIWVNNTIINGSQVWGGQP  96
        **********:* ******:***:*******:*********************.********

rat     VYPREPDDACIFPDGGPCPSGPKPPRRSFVYVWKTWGQYWQVLGGPESKLSIPTGHARLG 179
mouse   VYPQEPDDACVFPDGGPCPSGPKPPKRSFVYVWKTWGKYWQVLGGPVSRLSIATGHAKLG 179
human   VYPQETDDACIFPDGGPCPSGSWSQKRSFVYVWKTWGQYWQVLGGPVSGLSIGTGRAMLG 180
cyno    VYPQETDDACIFPDGGPCPSGPWSQKRSFVYVWKTWGQYWQVLGGPVSGLSIGTGRAMLG 156
        ***:*.*****:********** . .:*********** * *** **:* **

rat     THTMEVTVYHRRGSQSYVPLAHSSSTFTIT---------------------------- 209
mouse   THTMEVTVYHRRGSQSYVPLAHASSTFTTTDQVPFSVSVSQLQALDGETKHFLRNHPLIF 239
human   THTMEVTVYHRRGSRSYVPLAHSSSAFTITDQVPFSVSVSQLRALDGGNKHFLRNQPLTF 240
cyno    THTMEVTVYHRRGSRSYVPLAHSSSAFTITDQVPFSVSVSQLRALDGGNKHFLRNQPLTF 216
        **********:********:**:*****++++++++++++++  ++++  ++++++ ++ +

rat     ---------------------------------------------------------- 
mouse   ALQLHDPSGYLAEADLSYTWDFGDGTGTLISRALDVTHTYLESGSVTAQVVLQAAIPLVS 299
human   ALQLHDPSGYLAEADLSYTWDFGDSSGTLISRALVVTHTYLEPGPVTAQVVLQAAIPLTS 300
cyno    ALQLHDPSGYLAEADLSYTWDFGDSSGTLISRALVVTHTYLEPGPVTAQVVLQAAIPLTS 276
        ++++++++++++++++++++++++++++  ++++++++ +++++++ + ++++++++++++ +

rat     ---------------------------------------------------------- 
mouse   CGSSPVPGTTDGYMPTAEAPGTTSRQGTTTKVVGTTPGQMPTTQPSGTTVVQMPTTEVTA 359
human   CGSSPVPGTTDGHRPTAEAPNTTAGQVPTTEVVGTTPGQAPTAEPSGTTSVQVPTTEVIS 360
cyno    CGSSPVPGTTDGHRPTAEAPDTTAGRGPTTEVVGTTPGQVPTTQPSGTTSVQVPTTEVIS 336
        ++++++++++++  ++++++ ++      ++ ++++++++ ++  +++++ ++ +++++

rat   SEQ ID NO: 29
mouse SEQ ID NO: 31
human SEQ ID NO: 26
cyno  SEQ ID NO: 28
```

FIG. 9A

```
rat     ------------------------------------------------------------
mouse   TTSEQMLTSA-------------VIDTTLAEVSTTEGTGTTPTRPSGTTVA--------- 397
human   TAPVQMPTAESTGMTPEKVPVSEVMGTTLAEMSTPEATGMTPAEVSIVVLSGTTAAQVTT 420
cyno    TTPVQMPTAESTGTTPEKVPVSEVMGTTLAEMSTPEAIGMTPAEVSIVVPSGTTAAQVTT 396
        +   ++ +               +  +++++ ++ +  + ++   +

rat     -------------------------------GSVSRLLDDTDTIMLVKRQVPLDCVLYRY 238
mouse   -----------QATTTEGPDASPLLPTQSSTGSISPLLDDTDTIMLVKRQVPLDCVLYRY 446
human   TEWVETTARELPIPEPEGPDASSIMSTESITGSLGPLLDGTATLRLVKRQVPLDCVLYRY 480
cyno    TEWVETTAGELPTPEPEGPDTSSIMSTESITGSLGPLLDGTATLRLEKRQVPLDCVLYRY 456
                      ++++ +    + +  **:. ***.* *: * *************

rat     GSFSLTLDIVQGIESAEILQAVPSSEGDAFELTVSCRGGLPKEACMDISSPGCQPPAQRL 298
mouse   GSFSLALDIVQGIESAEILQAVPFSEGDAFELTVSCQGGLPKEACMDISSPGCQPPAQRL 506
human   GSFSVTLDIVQGIESAEILQAVPSGEGDAFELTVSCQGGLPKEACMEISSPGCQPPAQRL 540
cyno    GSFSVTLDIVQGIESAEILQVVPSSEGDAFELTVSCQGGLPTEACMEISSPGCQPPAQQL 516
        ****::***************.** .***********:****.****:***********:*

rat     CQPVPPSPDCQLVLHQILKGGLGTYCLNVSLADANSLAVASTQLVVPGQEGSLGQAPLLV 358
mouse   CQSVPPSPDCQLVLHQVLKGGSGTYCLNVSLADANSLAVASTQLVVPGQDGGLGQAPLLV 566
human   CQPVLPSPACQLVLHQILKGGSGTYCLNVSLADTNSLAVVSTQLIMPGQEAGLGQVPLIV 600
cyno    CQPVPPSPACQLVLYQILKGGLGTYCLNVSLADANSLAVVSTQLIVPGQEAGLGQAPLFV 576
        **.* *** *****:*:**** ***********:*****.****::***:..***.**:*

rat     GVLLVLVAVVLASLIYRHRLKKQD-SVSQTPHGSTHWLRLPPVFCARRLGESSPLLSGQQ 417
mouse   GILLVLVAVVLASLIHRHRLKKQG-SVSQMPHGSTHWLRLPPVFRARGLGENSPLLSGQQ 625
human   GILLVLMAVVLASLIYRRRLMKQDFSVPQLPHSSSHWLRLPRIFCSCPIGENSPLLSGQQ 660
cyno    GILLVLMAVVLASLIYRRRLMKQAFSIPQLPHGSSHWLRLPRIFRSCPIGENSPLLSGQE 636
        *:****:********:*:** **  *:.* **.*:****** :* :  :**.*******:

rat     V 418  SEQ ID NO: 29
mouse   V 626  SEQ ID NO: 31
human   V 661  SEQ ID NO: 26
cyno    V 637  SEQ ID NO: 28
        *
```

FIG. 9B


A
= 5E9v1-vcE, IV (3 mg/kg)
= 5E9v1-vcE, IV (3 mg/kg)
= 5E9v1-vcE, IV (8 mg/kg)
= 5E9v1-vcE, IV (10 mg/kg)
Individual Tumor Volume (mm³)
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
140
160
Day
ADC dosing
IV, once,3 mpk

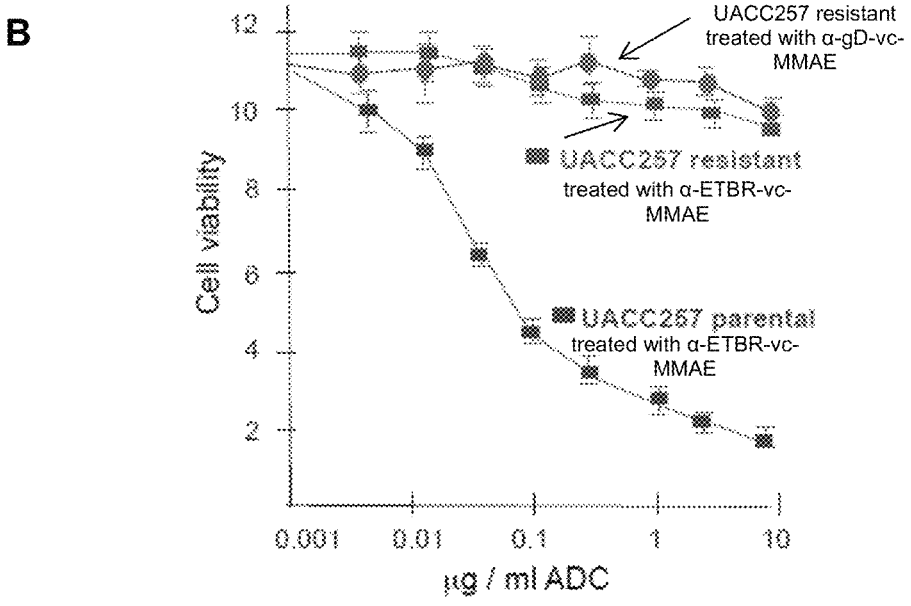

B
UACC257 resistant
treated with α-gD-vc-
MMAE
UACC257 resistant
treated with α-ETBR-vc-
MMAE
UACC257 parental
treated with α-ETBR-vc-
MMAE
Cell viability
12
10
8
6
4
2
0.001
0.01
0.1
1
10
μg / ml ADC

FIG. 10

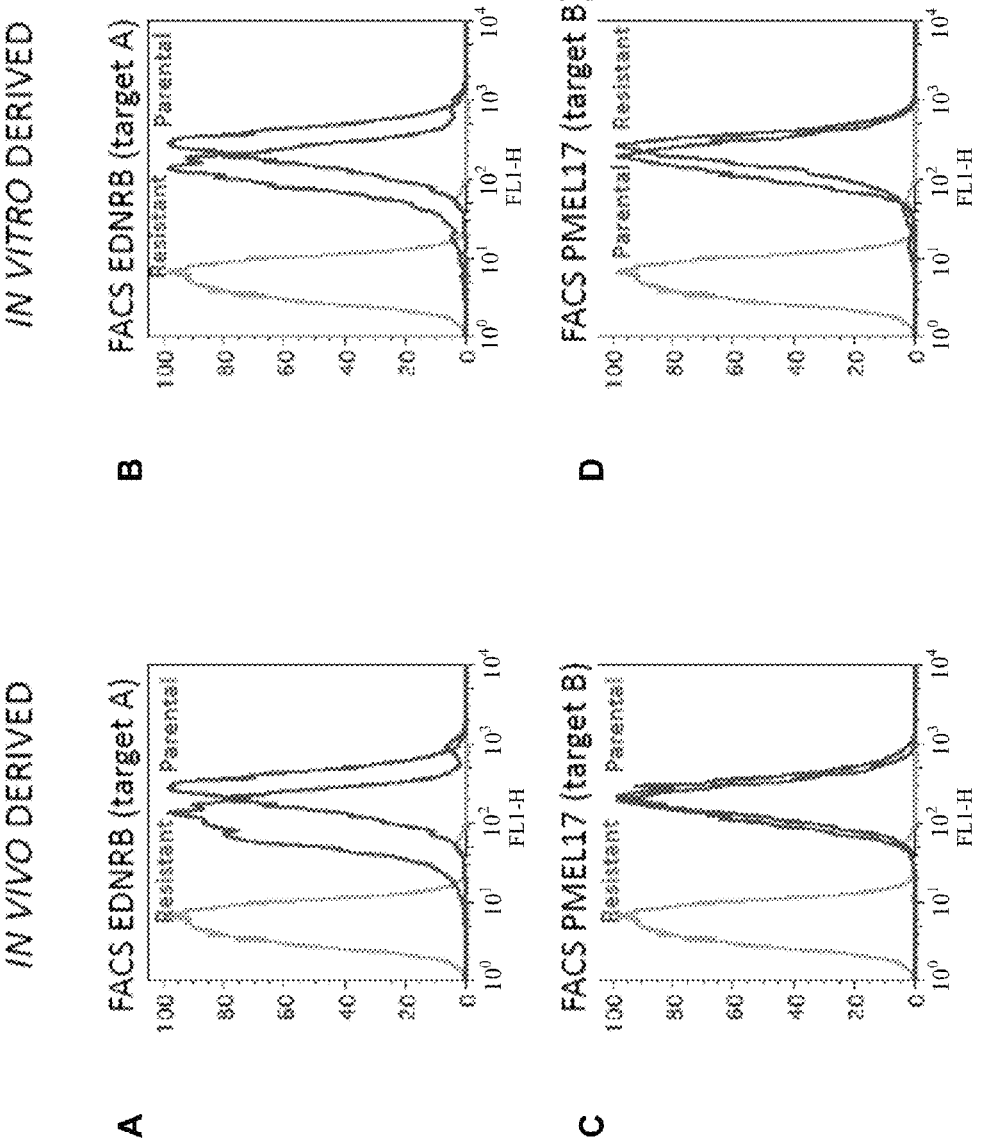

IN VIVO DERIVED
IN VITRO DERIVED
A
B
C
D
FACS EDNRB (target A)
FACS PMEL17 (target B)
Resistant
Parental
FL1-H

FIG. 11

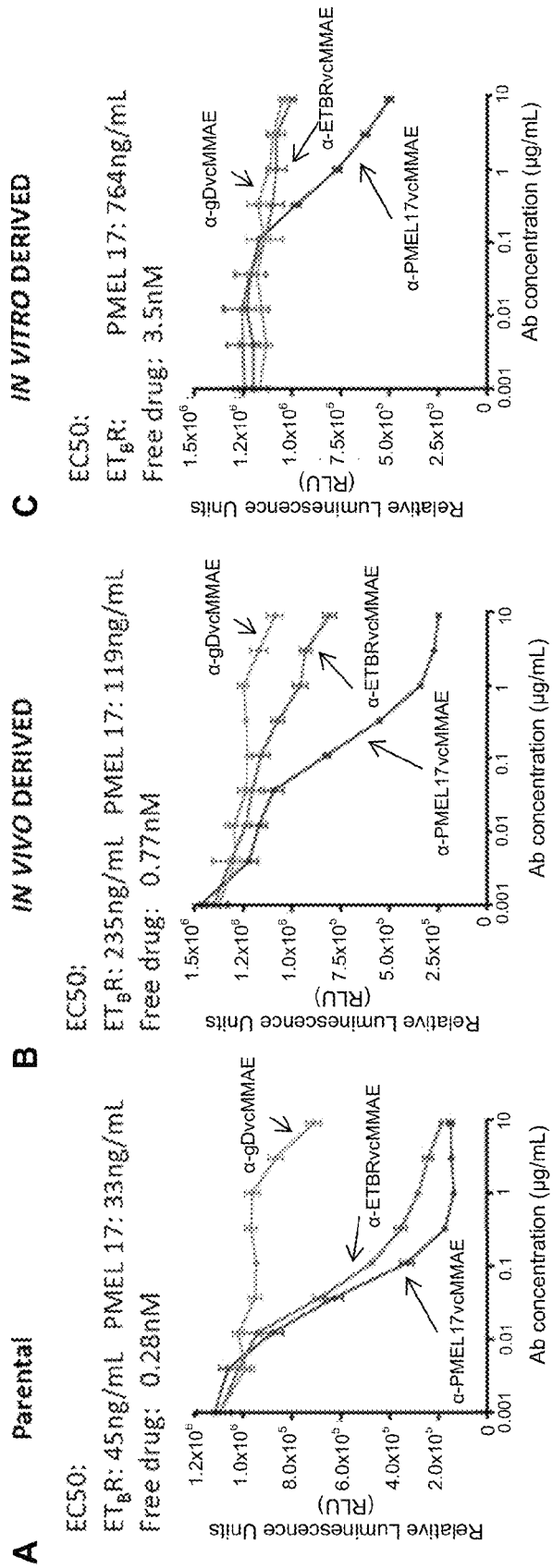

A
Parental
EC50:
ETBR: 45ng/mL PMEL 17: 33ng/mL
Free drug: 0.28nM
B
IN VIVO DERIVED
EC50:
ETBR: 235ng/mL PMEL 17: 119ng/mL
Free drug: 0.77nM
C
IN VITRO DERIVED
EC50:
ETBR: PMEL 17: 764ng/mL
Free drug: 3.5nM
Relative Luminescence Units (RLU)
Ab concentration (µg/mL)
α-gDvcMMAE
α-ETBRvcMMAE
α-PMEL17vcMMAE

FIG. 12

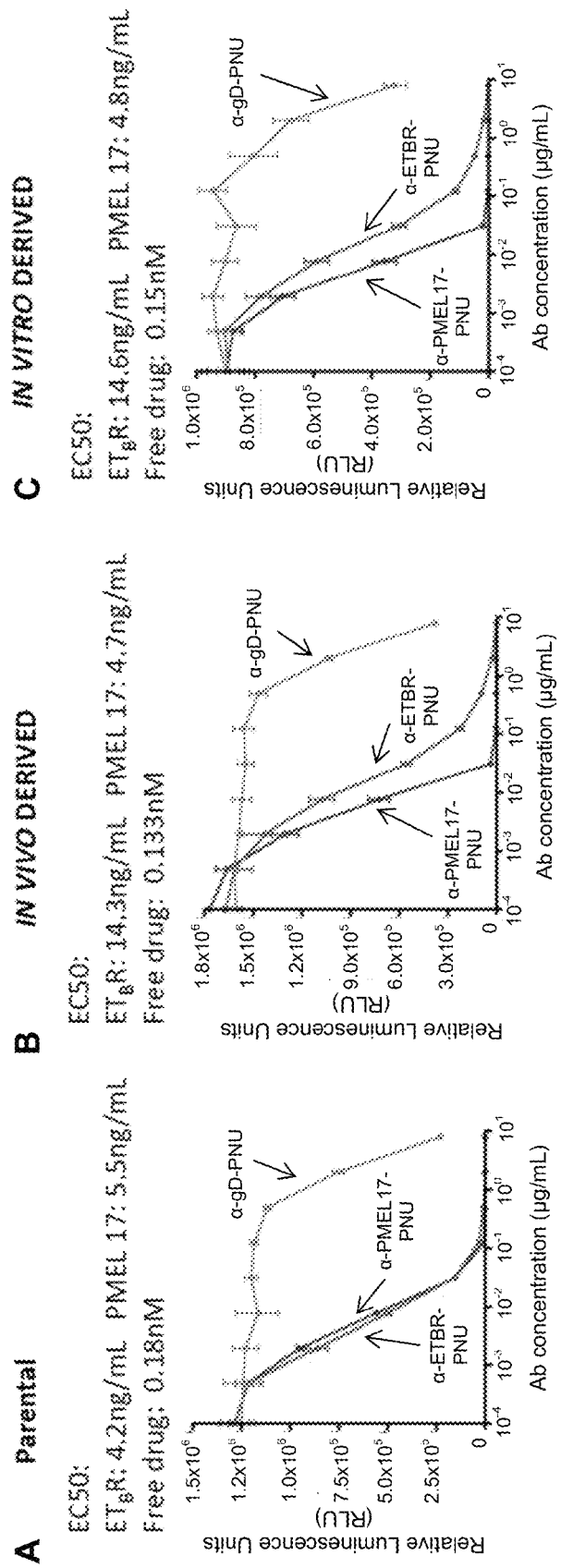

A
Parental
EC50:
ET_gR: 4.2ng/mL PMEL 17: 5.5ng/mL
Free drug: 0.18nM
B
IN VIVO DERIVED
EC50:
ET_gR: 14.3ng/mL PMEL 17: 4.7ng/mL
Free drug: 0.133nM
C
IN VITRO DERIVED
EC50:
ET_gR: 14.6ng/mL PMEL 17: 4.8ng/mL
Free drug: 0.15nM
Relative Luminescence Units (RLU)
Ab concentration (µg/mL)
α-gD-PNU
α-PMEL17-PNU
α-ETBR-PNU

FIG. 13

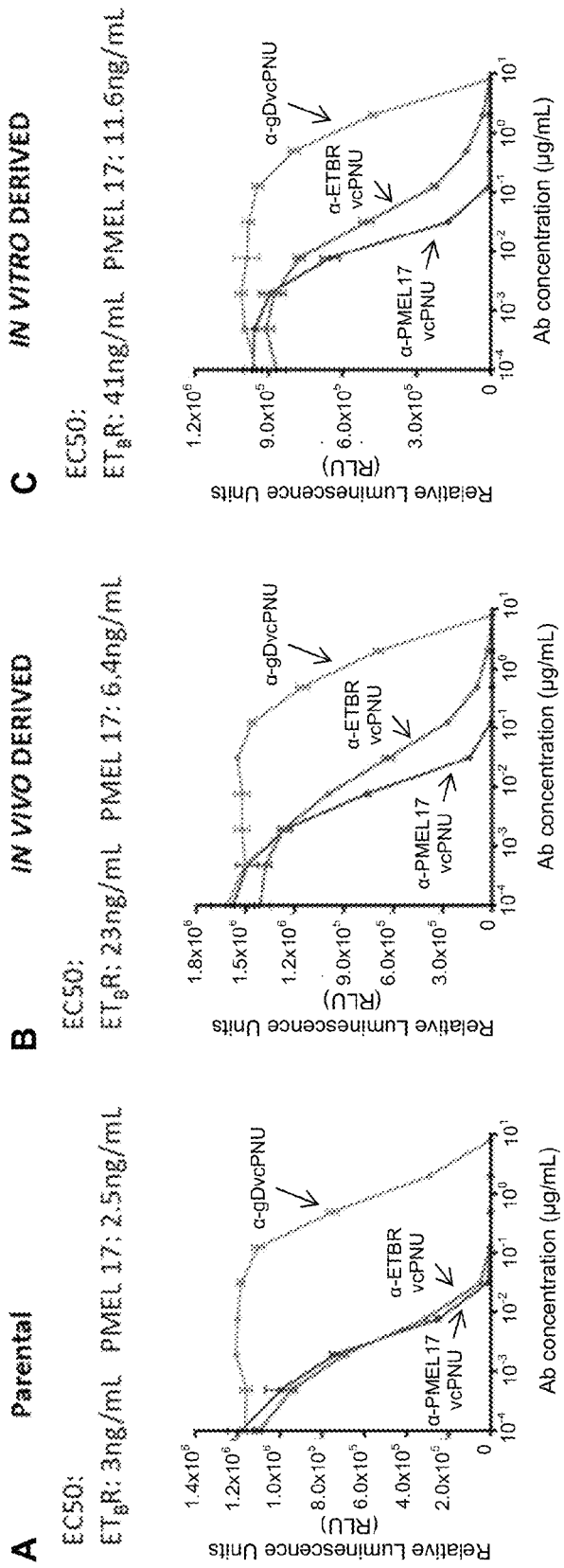
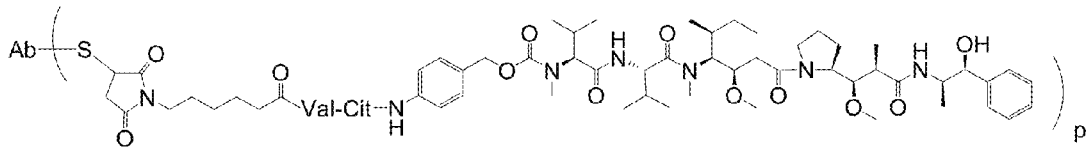
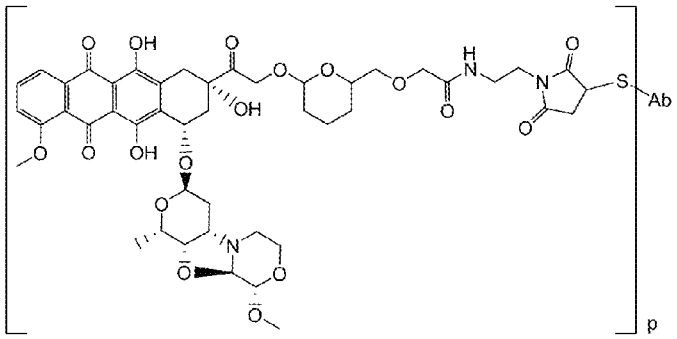

A
Parental
EC50:
ETBR: 3ng/mL PMEL 17: 2.5ng/mL
B
IN VIVO DERIVED
EC50:
ETBR: 23ng/mL PMEL 17: 6.4ng/mL
C
IN VITRO DERIVED
EC50:
ETBR: 41ng/mL PMEL 17: 11.6ng/mL
Relative Luminescence Units (RLU)
Ab concentration (µg/mL)
α-gDvcPNU
α-ETBR vcPNU
α-PMEL17 vcPNU

FIG. 14

A Ab-MC-val-cit-PAB-MMAE



B Ab-MC-acetal-PNU-159682



C Ab-MC-val-cit-PAB-PNU-159682

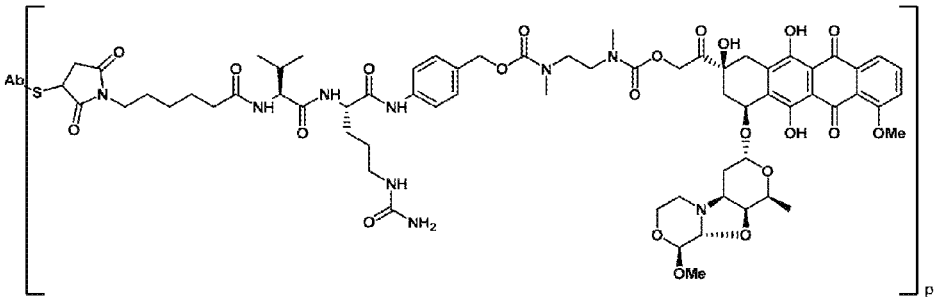
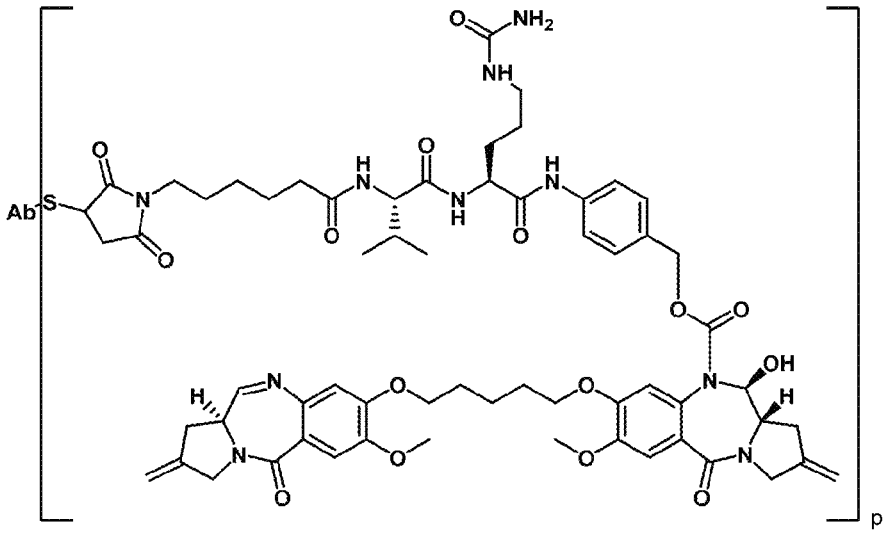


FIG. 15

D Ab-MC-val-cit-PAB-PBD



E Ab-PNU-159682

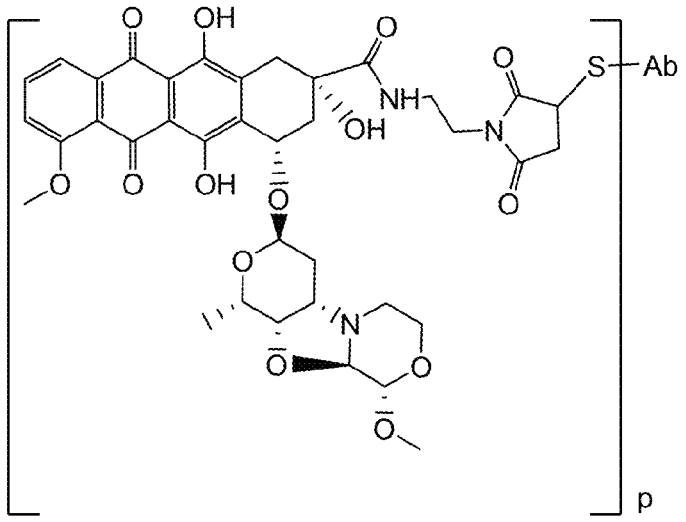


FIG. 15

ANTI-PMEL17 ANTIBODIES AND IMMUNOCONJUGATES

This application claims the benefit of U.S. Provisional Application No. 61/641,074, filed May 1, 2012, and U.S. Provisional Application No. 61/678,911, filed Aug. 2, 2012; each of which is incorporated by reference herein in its entirety for any purpose.

FIELD OF THE INVENTION

The present invention relates to anti-PMEL17 antibodies and immunoconjugates and methods of using the same.

BACKGROUND

Melanocyte protein PMEL17 is an integral membrane protein that undergoes export from the endoplasmic reticulum to the Golgi apparatus where it is glycosylated and ultimately trafficked to the melanosome. The specific route by which mature PMEL17 makes it way to the melanosome has been a subject of debate, however, it is apparent that some fraction of the protein is presented transiently at the cell surface prior to its entry into stage I melanosomes. Melanosomes are a specialized lysosome-related organelle that produce melanin pigments, which are deposited on fibrils composed of proteolytic fragments derived from the PMEL17 protein. The synthesis of melanin pigments is largely restricted to melanocytes and the post-mitotic pigment epithelium of the eye. Melanocytes uniquely express specialized genes required for pigment formation, some of which are maintained following their transformation to melanoma.

Human PMEL17 is a 661 amino acid protein (including the amino-terminal signal sequence), with a transmembrane region located at amino acids 596 and 616. PMEL17 undergoes complex processing, but at least a portion of the protein's lifecycle is spent on the cell surface.

There is a need in the art for agents that target PMEL17 for the diagnosis and treatment of PMEL17-associated conditions, such as cancer. The invention fulfills that need and provides other benefits.

SUMMARY

The invention provides anti-PMEL17 antibodies and immunoconjugates and methods of using the same.

In some embodiments, an isolated antibody that binds PMEL17 is provided. In some such embodiments, the antibody binds an epitope within amino acids 105 to 125 of SEQ ID NO: 26. In some such embodiments, the antibody binds to an epitope within amino acids 25 to 45 of SEQ ID NO: 26. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a human, humanized, or chimeric antibody. In some embodiments, the antibody is an antibody fragment that binds PMEL17. In some embodiments, PMEL17 is human PMEL17. In some embodiments, human PMEL17 has the sequence of SEQ ID NO: 26 or SEQ ID NO: 27. In some embodiments, the antibody is an IgG1, IgG2a or IgG2b antibody.

In some embodiments, the antibody comprises: a) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4; or b) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; or c) HVR-H3, HVR-L3, and HVR-H2 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In some embodiments, the antibody comprises: a) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5; or b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15; or c) HVR-H1, HVR-H2, and HVR-H3 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In some embodiments, the antibody comprises: a) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or c) HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 of the antibody produced by hybridoma 7509 (31D1.6.7) having ATCC Accession No. PTA-12862.

In some embodiments, the antibody comprises: a) (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or b) (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or c) HVR-L1, HVR-L2, and HVR-L3 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In some embodiments, the antibody comprises: a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1 or 50; or b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 2; or c) a VH sequence as in (a) and a VL sequence as in (b); or d) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9 or 49; or e) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10; or f) a VH sequence as in (d) and a VL sequence as in (e); or g) a VH sequence having at least 95% sequence identity to the VH sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862; or h) a VL sequence having at least 95% sequence identity to the VL sequence of the antibody produced by hybridoma 7509 (31D1.6.7) having ATCC Accession No. PTA-12862; or i) a VH sequence as in (g) and a VL sequence as in (h).

In some embodiments, the antibody comprises a VH sequence having the amino acid sequence of SEQ ID NO: 1 or 50, a VH sequence having the amino acid sequence of SEQ ID NO: 9 or 49, or a VH sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In some embodiments, the antibody comprises a VL sequence having the amino acid sequence of SEQ ID

NO:2, a VL sequence having the amino acid sequence of SEQ ID NO: 10, or a VL sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In some embodiments, the antibody comprises (a) a VH sequence having the amino acid sequence of SEQ ID NO: 1 or 50 and a VL sequence having the amino acid sequence of SEQ ID NO: 2; or (b) a VH sequence having the amino acid sequence of SEQ ID NO: 9 or 49 and a VL sequence having the amino acid sequence of SEQ ID NO: 10; or (c) a VH sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862 and a VL sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

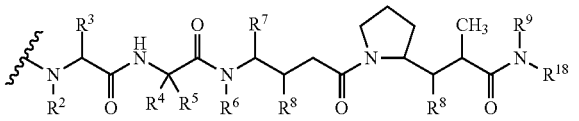
In some embodiments, an isolated antibody that binds PMEL17 is provided, wherein the antibody comprises: a) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21; or b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22; or c) (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24, (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25; or d) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24, (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25; or e) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11 or 51; or f) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 12; or g) a VH sequence as in (e) and a VL sequence as in (f); or h) a VH sequence having the amino acid sequence of SEQ ID NO: 11 or 51; or i) a VL sequence having the amino acid sequence of SEQ ID NO: 12; or j) a VH sequence as in (h) and a VL sequence as in (i). In some embodiments, the antibody is an IgG1, IgG2a or IgG2b antibody.

In some embodiments, an isolated nucleic acid encoding any of the foregoing antibodies is provided. In some embodiments, a host cell comprising the nucleic acid is provided. In some embodiments, a method of producing an antibody is provided, comprising culturing the host cell so that the antibody is produced.

In some embodiments, an immunoconjugate is provided. In some such embodiments, the immunoconjugate comprises any of the foregoing antibodies and a cytotoxic agent. In some embodiments, the immunoconjugate has the formula Ab-(L-D)p, wherein: (a) Ab is the antibody of any one of claim **1** to **16**; (b) L is a linker; (c) D is a drug selected from a maytansinoid, an auristatin, a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative; and (d) p ranges from 1-8.

In some embodiments, D is an auristatin. In some such embodiments, D has formula D_E

D_E

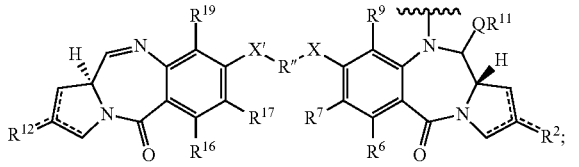


and wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^5 is H, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O—CH_3, OH, and H; R^9 is H; and R^{18} is —$C(R^8)_2$—$C(R^8)_2$-aryl.

In some embodiments, the drug (D) is MMAE.

In some embodiments, D is a pyrrolobenzodiazepine of Formula A:

A



wherein the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R^2 is independently selected from H, OH, =O, =CH2, CN, R, OR, =CH—R^D, =$C(R^D)_2$, O—SO_2—R, CO_2R and COR, and optionally further selected from halo or dihalo, wherein

R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

Q is independently selected from O, S and NH;

R^{11} is either H, or R or, where Q is O, SO_3M, where M is a metal cation;

R and R' are each independently selected from optionally substituted C_{1-8} alkyl, C_{3-8} heterocyclyl and C_{5-20} aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

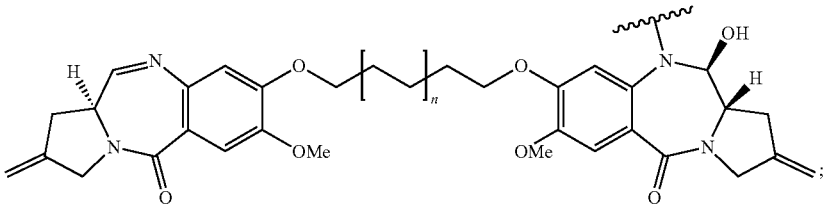
R^{12}, R^{16}, R^{19} and R^{17} are as defined for R^2, R^6, R^9 and R^7 respectively;

R" is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms and/or aromatic rings that are optionally substituted; and

X and X' are independently selected from O, S and N(H).

In some such embodiments, D has the structure:

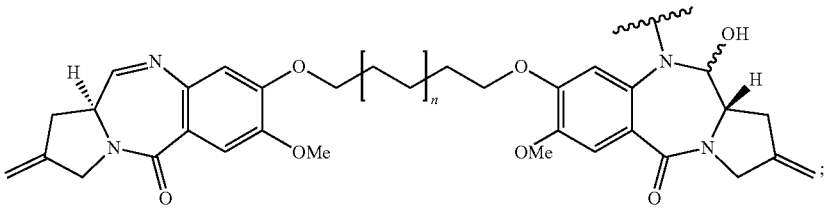
A(II)



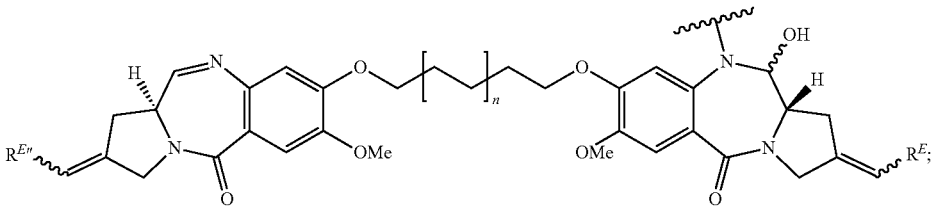
wherein n is 0 or 1.

In some embodiments, D has a structure selected from:

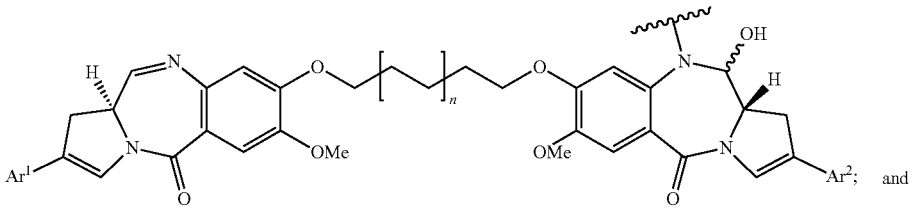
A(I)



A(III)

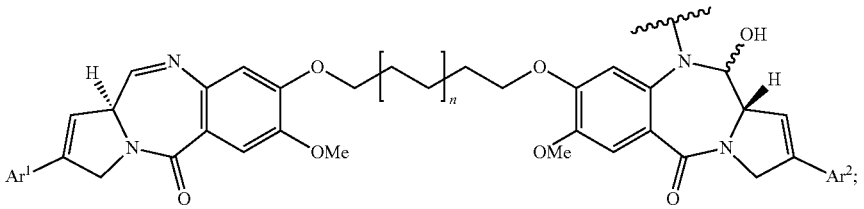


A(IV)



and

A(V)

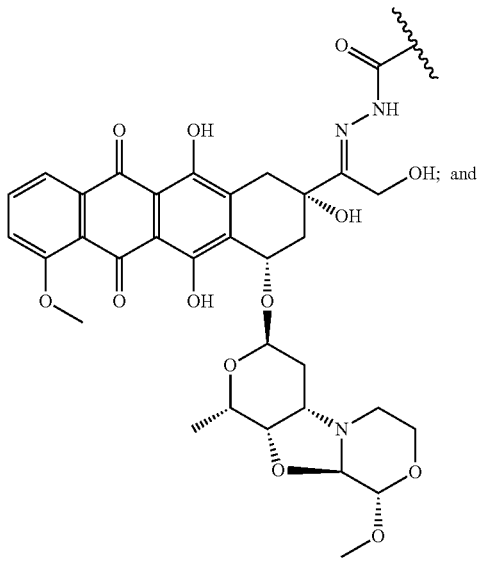


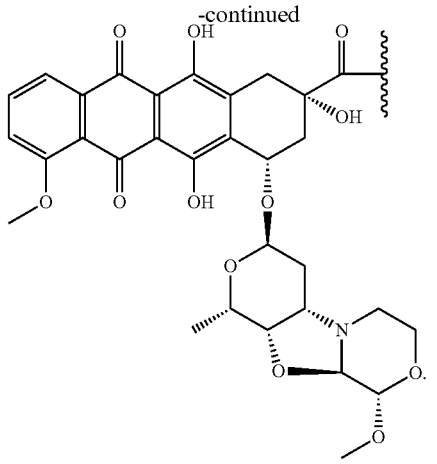
wherein R^E and $R^{E''}$ are each independently selected from H or R^D, wherein R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

wherein Ar^1 and Ar^2 are each independently optionally substituted C_{5-20} aryl; and

wherein n is 0 or 1.

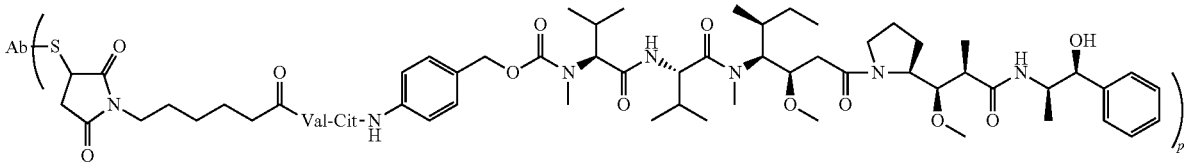
In some embodiments, D is a nemorubicin derivative. In some such embodiments, D has a structure selected from:





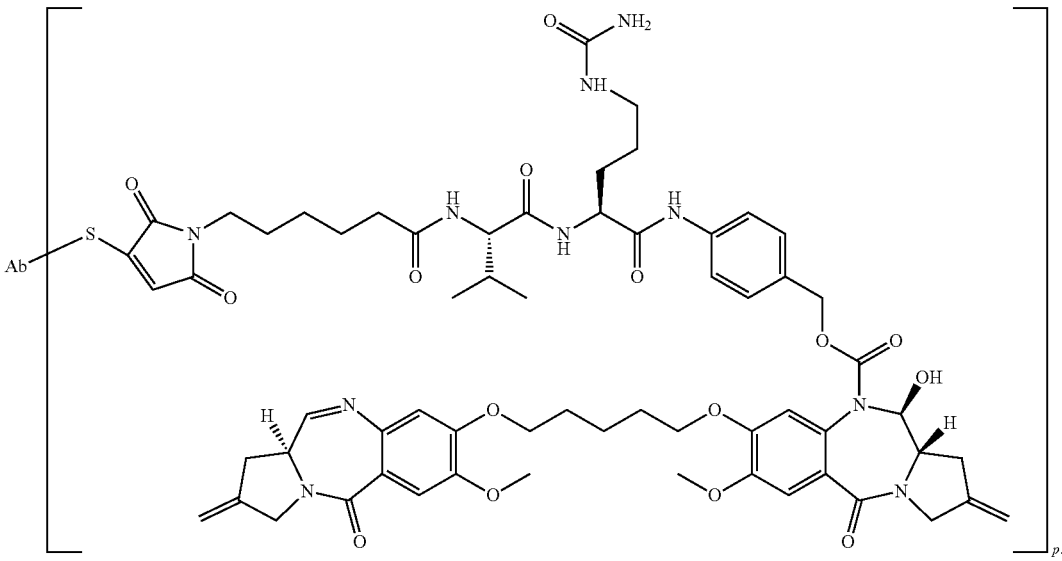
In some embodiments, the linker is cleavable by a protease. In some such embodiments, the linker comprises a val-cit dipeptide or a Phe-Lys dipeptide. In some embodiments, the linker is acid-labile. In some such embodiments, the linker comprises hydrazone.

In some embodiments, an immunoconjugate has the formula:

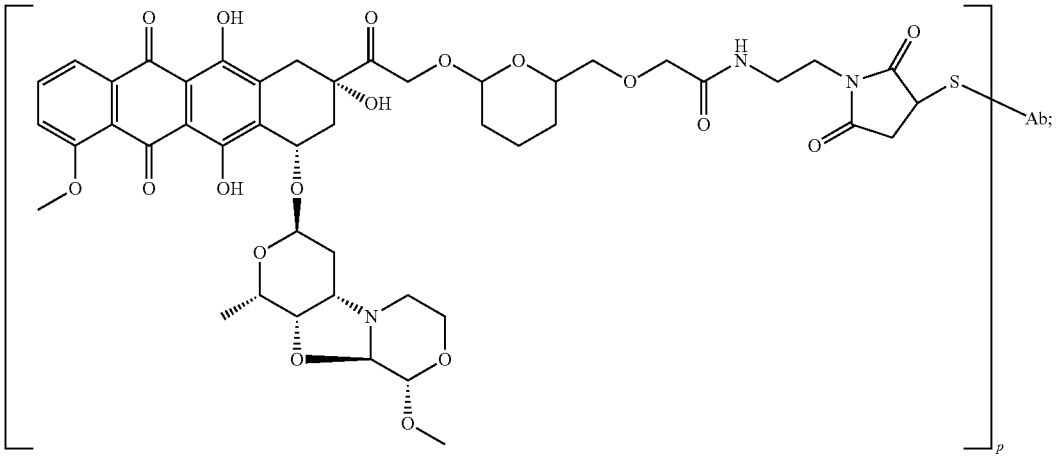


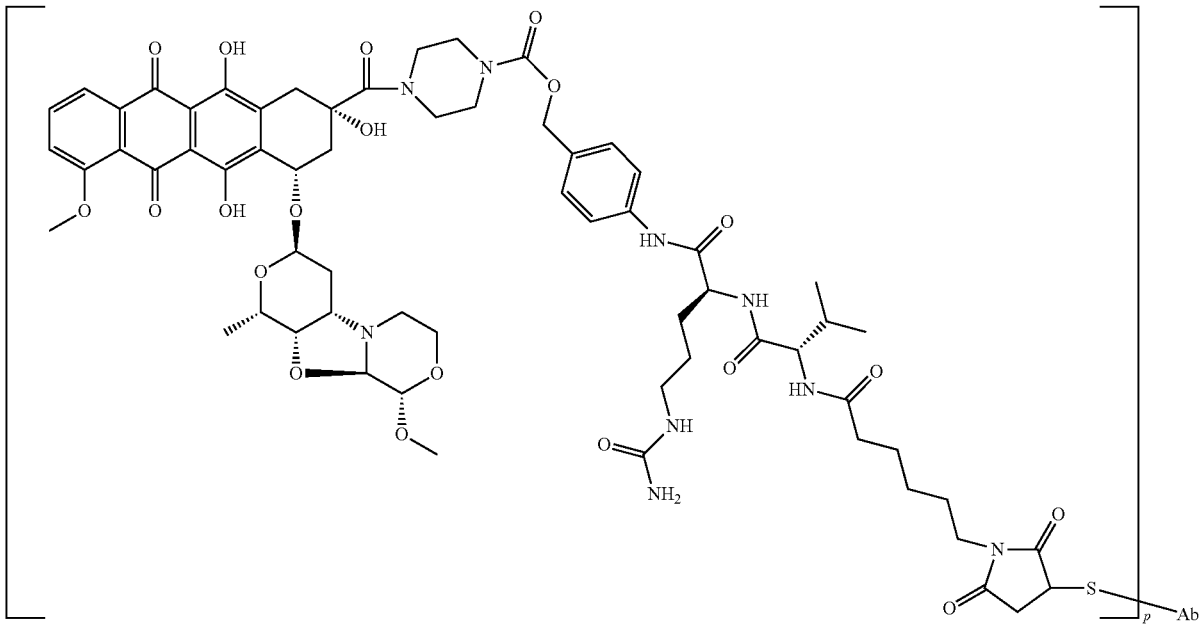
wherein S is a sulfur atom.

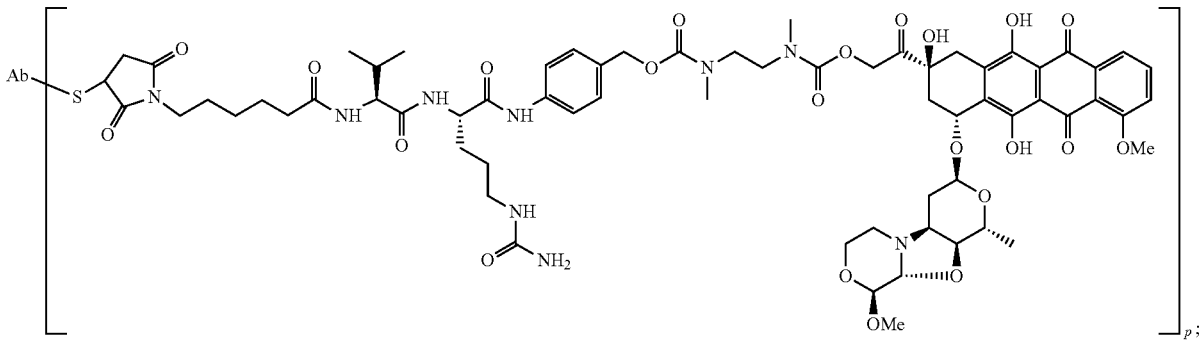
In some embodiments, an immunoconjugate has the formula:



In some embodiments, an immunoconjugate has a formula selected from:







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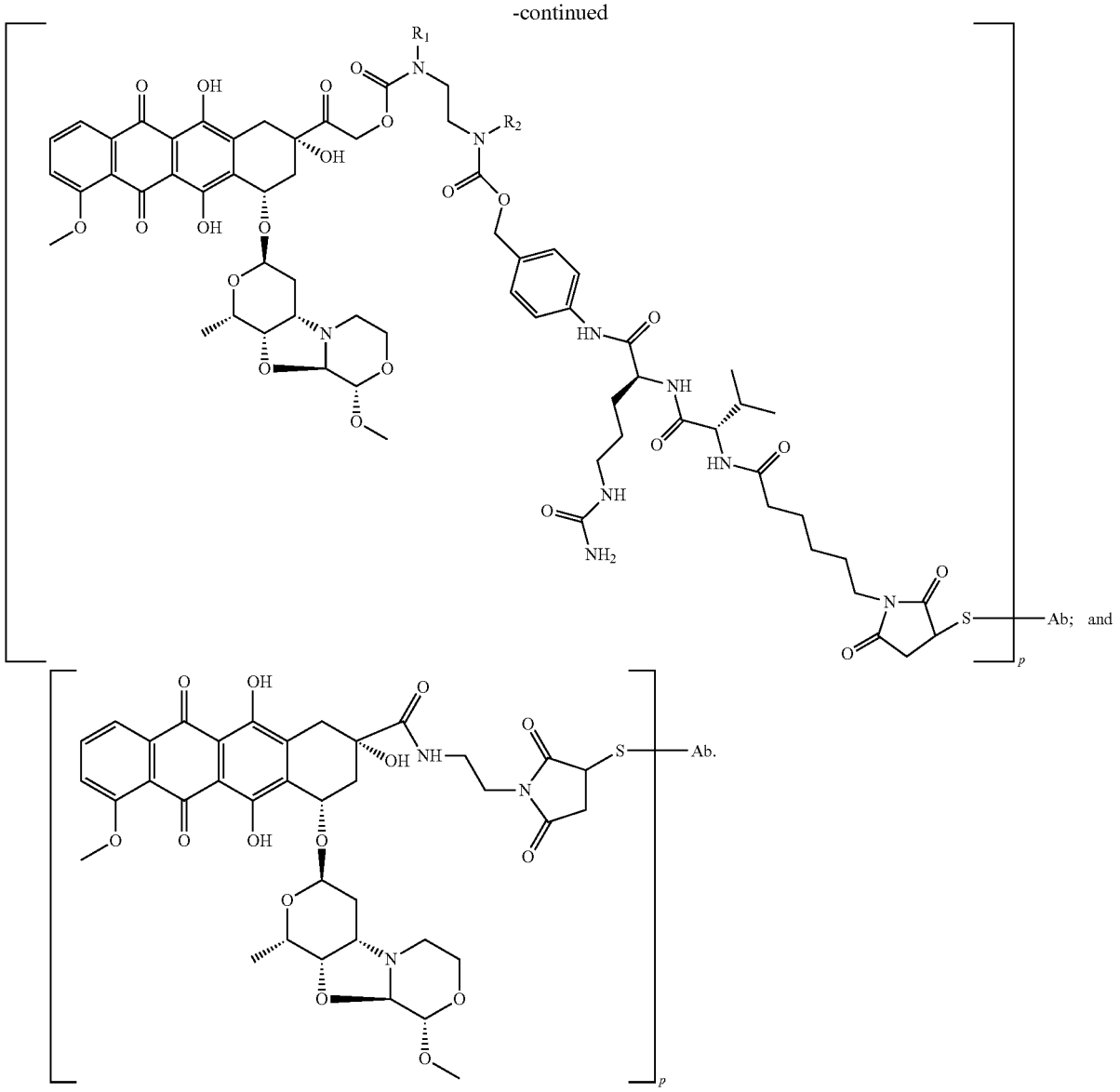


In some embodiments of the foregoing immunoconjugates, p ranges from 2-5.

In some embodiments, an immunoconjugate is provided, wherein the immunoconjugate comprises an antibody that comprises: a) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or c) HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In some embodiments, an immunoconjugate is provided, wherein the immunoconjugate comprises an antibody that comprises (a) a VH sequence having the amino acid sequence of SEQ ID NO: 1 or 50 and a VL sequence having the amino acid sequence of SEQ ID NO:2; or (b) a VH sequence having the amino acid sequence of SEQ ID NO: 9 or 49 and a VL sequence having the amino acid sequence of SEQ ID NO: 10; or (c) a VH sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862 and a VL sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In some embodiments, pharmaceutical formulations are provided. In some such embodiments, the pharmaceutical formulation comprises an antibody described herein and a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical formulation further comprises an additional therapeutic agent.

In some embodiments, methods of treating an individual having a PMEL17-positive cancer are provided. In some such embodiments, the method comprises administering to the individual an effective amount of an immunoconjugate comprising an antibody that binds to PMEL17. In some such embodiments, the method comprises administering to the individual an effective amount of an immunoconjugate comprising an antibody that binds to the extracellular domain of PMEL17. In some such embodiments, the method comprises administering to the individual an effective amount of an immunoconjugate comprising an antibody described herein. In some embodiments, the PMEL17-positive cancer is melanoma. In some embodiments, a method of treating an individual having a PMEL17-positive cancer further comprises administering an additional therapeutic agent to the individual.

In some embodiments, methods of treating an individual having a PMEL17-positive cancer are provided, wherein the PMEL17-positive cancer is resistant to a first therapeutic. In some embodiments, the method comprises administering to the individual an effective amount of an immunoconjugate comprising an antibody that binds to PMEL17. In some embodiments, the PMEL17-positive cancer is melanoma. In some embodiments, the first therapeutic comprises a first antibody that binds an antigen other than PMEL17. In some embodiments, the first therapeutic is a first immunoconjugate comprising a first antibody that binds an antigen other than PMEL17 and a first cytotoxic agent. In some embodiments, the first antibody binds an antigen selected from endothelin B receptor (ETBR), tyrosinase-related protein 1 (TYRP1), cytotoxic T lymphocyte antigen 4 (CTLA-4), and glycoprotein NMB (GPNMB). In some embodiments, the first antibody binds ETBR. In some embodiments, the first antibody is hu5E9.v1. In some embodiments, the first immunoconjugate is hu5E9.v1-MC-val-cit-PAB-MMAE. In some embodiments, the first cytotoxic agent and the cytotoxic agent of the immunoconjugate comprising an antibody that binds to PMEL17 are different. In some such embodiments, the first cytotoxic agent is MMAE and the cytotoxic agent of the immunoconjugate comprising an antibody that binds to PMEL17 is selected from a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative. In some embodiments, the cytotoxic agent of the immunoconjugate comprising an antibody that binds to PMEL17 is selected from a pyrrolobenzodiazepine and a nemorubicin derivative.

In some embodiments, a method of treating an individual with PMEL17-positive cancer is provided, wherein the method comprises administering to the individual an effective amount of a first immunoconjugate described herein in combination with a second immunoconjugate comprising an antibody that binds ETBR. In some embodiments, the antibody that binds ETBR comprises an HVR H1 comprising a sequence of SEQ ID NO: 33, an HVR H2 comprising a sequence of SEQ ID NO: 34, an HVR H3 comprising a sequence of SEQ ID NO: 35, an HVR L1 comprising a sequence of SEQ ID NO: 36, an HVR L2 comprising a sequence of SEQ ID NO: 37, and an HVR L3 comprising a sequence of SEQ ID NO: 38. In some embodiments, the antibody that binds ETBR comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 40 and a light chain variable region comprising the sequence of SEQ ID NO: 39. In some embodiments, the first immunoconjugate comprises a cytotoxic agent selected from an auristatin, a pyrrolobenzodiazepine, and a nemorubicin derivative, and the second immunoconjugate comprises a cytotoxic agent selected from an auristatin, a pyrrolobenzodiazepine, and a nemorubicin derivative. In some embodiments, the first immunoconjugate comprises a cytotoxic agent selected from a pyrrolobenzodiazepine and a nemorubicin derivative, and the second immunoconjugate comprises an auristatin. In some embodiments, the second immunoconjugate comprises MMAE. In some such embodiments, the second immunoconjugate comprises a linker-drug portion comprising MC-val-cit-PAB-MMAE. In some embodiments, the second immunoconjugate is hu5E9.v1-MC-val-cit-PAB-MMAE. In any of the foregoing embodiments, the PMEL17-positive cancer may be melanoma. In some embodiments, the PMEL17-positive cancer is also ETBR-positive.

In some embodiments, a method of inhibiting proliferation of PMEL17-positive cell is provided. In some such embodiments, the method comprises exposing the cell to an immunoconjugate described herein under conditions permissive for binding of the immunoconjugate to PMEL17 on the surface of the cell, thereby inhibiting proliferation of the cell. In some embodiments, the cell is a melanoma cell.

In some embodiments, an antibody that binds to PMEL17 conjugated to a label is provided. In some embodiments, the label is a positron emitter. In some such embodiments, the positron emitter is ^{89}Zr.

In some embodiments, a method of detecting human PMEL17 in a biological sample is provided. In some embodiments, the method comprises contacting the biological sample with an anti-PMEL17 antibody described herein under conditions permissive for binding of the anti-PMEL17 antibody to a naturally occurring human PMEL17. In some embodiments, the method further comprises detecting whether a complex is formed between the anti-PMEL17 antibody and a naturally occurring human PMEL17 in the biological sample. In some embodiments, the anti-PMEL17 antibody is the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In some embodiments, the antibody comprises a) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or c) HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 of the antibody produced by hybridoma 7509 (31D1.6.7) having ATCC Accession No. PTA-12862In some embodiments, the biological sample is a melanoma sample.

In some embodiments, a method for detecting a PMEL17-positive cancer is provided. In some such embodiments, the method comprises (i) administering a labeled anti-PMEL17 antibody to a subject having or suspected of having a PMEL17-positive cancer, wherein the labeled anti-PMEL17 antibody comprises an anti-PMEL17 antibody described herein, and (ii) detecting the labeled anti-PMEL17 antibody in the subject, wherein detection of the labeled anti-PMEL17 antibody indicates a PMEL17-positive cancer in the subject. In some such embodiments, the labeled anti-PMEL17 antibody comprises an anti-PMEL17 antibody conjugated to a positron emitter. In some embodiments, the positron emitter is ^{89}Zr.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows (A) a graphic representation of the levels of human PMEL17 gene expression in various tissues, and (B) a graphic representation of the levels of human PMEL17 in various human cell lines, as described in Example A.

FIGS. **2**A and **2**B show alignments of the (A) light chain and (B) heavy chain variable regions sequences for antibodies 8G3, 17A9, and 15F2.

FIGS. **3**A to **3**D shows epitope mapping of 77E6 (A and B), 17A9 (C and D), and 31D1 (D), as described in Example D.

FIG. **4** shows internalization of 17A9 in melanoma cells, as described in Example G.

FIG. **5** shows killing of various PMEL17+ melanoma cell lines by increasing concentrations of 17A9 ADC, as described in Example I.

FIG. **6** shows (A) distribution of 31D1 staining in normal skin, (B) 17A9 staining of normal melanocytes and melanoma cell line SK-MEL-5 by FACS, and (C) the IC_{50} of 17A9 on normal melanocytes is nearly 100-fold higher than the IC_{50} of 17A9 on melanoma cell lines, as described in Example J.

FIG. **7** shows (A) exemplary staining for each level of PMEL17 staining and (B) IHC scores for a panel of human melanoma samples, as described in Example K.

FIG. **8** shows efficacy of 17A9 ADC in an SK-MEL-23 cell line xenograft, as described in Example L.

FIGS. **9**A and **9**B show an alignment of human, mouse, rat, and cynomolgus monkey PMEL17, as described in Example F.

FIG. **10** shows (A) tumor volume over time in mice inoculated with UACC-257X2.2 melanoma cells and administered varying doses of anti-ETBR-vc-MMAE ("5E9v1-vcE"), and (B) parental and resistant UACC-257X2.2 cells grown in vitro in the presence of increasing concentrations of anti-ETBR-vc-MMAE ADC, as described in Example M.

FIG. **11** shows expression of ETBR (A and B; also referred to as "EDNRB") and PMEL17 (C and D) in parental and resistant UACC-257X2.2 cells derived in vivo (A and C) and in vitro (B and D), as described in Example M.

FIG. **12** shows sensitivity of (A) parental UACC-257X2.2 cells, (B) in vivo derived resistant UACC-257X2.2 cells, and (C) in vitro derived resistant UACC-257X2.2 cells to increasing concentrations of anti-ETBR-vc-MMAE, anti-PMEL17-vc-MMAE, and anti-gD-vc-MMAE, as described in Example M.

FIG. **13** shows sensitivity of (A) parental UACC-257X2.2 cells, (B) in vivo derived resistant UACC-257X2.2 cells, and (C) in vitro derived resistant UACC-257X2.2 cells to increasing concentrations of anti-ETBR-PNU, anti-PMEL17-PNU, and anti-gD-PNU, as described in Example M.

FIG. **14** shows sensitivity of (A) parental UACC-257X2.2 cells, (B) in vivo derived resistant UACC-257X2.2 cells, and (C) in vitro derived resistant UACC-257X2.2 cells to increasing concentrations of anti-ETBR-vc-PNU, anti-PMEL17-vc-PNU, and anti-gD-vc-PNU, as described in Example M.

FIG. **15** shows the structures of various antibody-drug conjugates, including (A) Ab-MC-val-cit-PAB-MMAE; (B) Ab-MC-acetal-PNU-159682; (C) Ab-MC-val-cit-PAB-PNU-159682; (D) Ab-MC-val-cit-PAB-PBD; and (E) Ab-PNU-159682.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

I. Definitions

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

"Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

The terms "anti-PMEL17 antibody" and "an antibody that binds to PMEL17" refer to an antibody that is capable of binding PMEL17 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting PMEL17. In one embodiment, the extent of binding of an anti-PMEL17 antibody to an unrelated, non-PMEL17 protein is less than about 10% of the binding of the antibody to PMEL17 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to PMEL17 has a dissociation constant (Kd) of ≤1 μM, ≤100 nM, ≤10 nM, ≤5 Nm, ≤4 nM, ≤3 nM, ≤2 nM, ≤1 nM, ≤0.1 nM, ≤0.01 nM, or ≤0.001 nM (e.g., 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-PMEL17 antibody binds to an epitope of PMEL17 that is conserved among PMEL17 from different species.

The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody and that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically charac-

terized by unregulated cell growth/proliferation. Examples of cancer include, but are not limited to, melanoma, carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ϵ, γ, and μ, respectively.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{2}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omega1I (see, e.g., Agnew, Chem. Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., paclitaxel (TAXOL®; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), and docetaxel (TAXOTERE®; Rhone-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; CVP, an abbreviation for a combined therapy of cyclophosphamide, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

"Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: Clq binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "epitope" refers to the particular site on an antigen molecule to which an antibody binds.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The term "glycosylated forms of PMEL17" refers to naturally occurring forms of PMEL17 that are post-translationally modified by the addition of carbohydrate residues.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda Md. (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH(H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H, 50-65 of H2, and 95-102 of H3. (Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991).) With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., supra.

An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

An "isolated antibody" is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An "isolated nucleic acid" refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated nucleic acid encoding an anti-PMEL17 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "PMEL17," as used herein, refers to any native PMEL17. The term includes PMEL17 from any vertebrate source, including mammals such as primates (e.g. humans and cynomolgus monkeys) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed PMEL17, as well as any form of PMEL17 that results from processing in the cell. The term also includes naturally occurring variants of PMEL17, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human PMEL17 preproprotein, with signal sequence (amino acids 1-24) is shown in SEQ ID NO: 26. The amino acid sequence of an exemplary human PMEL17 proprotein is shown in SEQ ID NO: 27. The predicted sequence of an exemplary cynomolgus monkey PMEL17 (without signal sequence) is shown in SEQ ID NO: 28. The amino acid sequences for exemplary rat PMEL17 with signal sequence (amino acids 1-25) and without signal sequence are shown in SEQ ID NOs: 29 and 30, respectively. The amino acid sequences for exemplary mouse PMEL17 with signal sequence (amino acids 1-25) and without signal sequence are shown in SEQ ID NOs: 31 and 32, respectively.

The term "PMEL17-positive cancer" refers to a cancer comprising cells that express (including transiently express) PMEL17 on their surface.

The term "PMEL17-positive cell" refers to a cell that expresses PMEL17 on its surface.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N— to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated

otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology,* 6^{th} ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

"Alkyl" is C_1-C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, —CH_3), ethyl (Et, —CH_2CH_3), 1-propyl (n-Pr, n-propyl, —$CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, —$CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, —$CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (1-Bu, i-butyl, —$CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, —$CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, —$C(CH_3)_3$), 1-pentyl (n-pentyl, —$CH_2CH_2CH_2CH_2CH_3$), 2-pentyl (—$CH(CH_3)CH_2CH_2CH_3$), 3-pentyl (—$CH(CH_2CH_3)_2$), 2-methyl-2-butyl (—$C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl (—$CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl (—$CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl (—$CH_2CH(CH_3)CH_2CH_3$), 1-hexyl (—$CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl (—$CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl (—$CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl (—$C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl (—$CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl (—$CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl (—$C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl (—$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl (—$C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl (—$CH(CH_3)C(CH_3)_3$.

The term "C_1-C_8 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 8 carbon atoms. Representative "C_1-C_8 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl and -n-decyl; while branched C_1-C_8 alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, unsaturated C_1-C_8 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, 3-hexylacetylenyl, -propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl, -3-methyl-1 butynyl. A C_1-C_8 alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, —C_1-C_8 alkyl, —O—(C_1-C_8 alkyl), -aryl, —C(O)R', —OC(O)R', —C(O)OR, —$C(O)NH_2$, —C(O)NHR', —$C(O)N(R')_2$ —NHC(O)R', —SO_3R', —$S(O)_2R'$, —S(O)R', —OH, -halogen, —N_3, —NH_2, —NH(R'), —$N(R')_2$ and —CN; where each R' is independently selected from H, —C_1-C_8 alkyl and aryl.

The term "C_1-C_{12} alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 12 carbon atoms. A C_1-C_{12} alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, —C_1-Csalkyl, —O—(C_1-C_8 alkyl), -aryl, —C(O)R', —OC(O)R', —C(O)OR, —$C(O)NH_2$, —C(O)NHR', —$C(O)N(R')_2$—NHC(O)R', —SO_3R', —$S(O)_2R'$, —S(O)R', —OH, -halogen, —N_3, —NH_2, —NH(R'), —$N(R')_2$ and —CN; where each R' is independently selected from H, —C_1-C_8 alkyl and aryl.

The term "C_1-C_6 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 6 carbon atoms. Representative "C_1-C_6 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl,—and n-hexyl; while branched C_1-C_6 alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, and 2-methylbutyl; unsaturated C_1-C_6 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, and -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, and 3-hexyl. A C_1-C_6 alkyl group can be unsubstituted or substituted with one or more groups, as described above for C_1-C_8 alkyl group.

The term "C_1-C_4 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 4 carbon atoms. Representative "C_1-C_4 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl; while branched C_1-C_4 alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl; unsaturated C_1-C_4 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, and -isobutylenyl. A C_1-C_4 alkyl group can be unsubstituted or substituted with one or more groups, as described above for C_1-C_8 alkyl group.

"Alkoxy" is an alkyl group singly bonded to an oxygen. Exemplary alkoxy groups include, but are not limited to, methoxy (—OCH_3) and ethoxy (—OCH_2CH_3). A "C_1-C_5 alkoxy" is an alkoxy group with 1 to 5 carbon atoms. Alkoxy groups may can be unsubstituted or substituted with one or more groups, as described above for alkyl groups.

"Alkenyl" is C_2-C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp^2 double bond. Examples include, but are not limited to: ethylene or vinyl (—CH═CH_2), allyl (—CH_2CH═CH_2), cyclopentenyl (—C_5H_7), and 5-hexenyl (—$CH_2CH_2CH_2CH_2$CH═CH_2). A "C_2-C_8 alkenyl" is a hydrocarbon containing 2 to 8 normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp^2 double bond.

"Alkynyl" is C_2-C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp triple bond. Examples include, but are not limited to: acetylenic (—C≡CH) and propargyl (—CH_2C≡CH). A "C_2-C_8 alkynyl" is a hydrocarbon containing 2 to 8 normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp triple bond.

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene (—CH_2—) 1,2-ethyl (—CH_2CH_2—), 1,3-propyl (—$CH_2CH_2CH_2$—), 1,4-butyl (—$CH_2CH_2CH_2CH_2$—), and the like.

A "C_1-C_{10} alkylene" is a straight chain, saturated hydrocarbon group of the formula —$(CH_2)_{1\text{-}10}$—. Examples of a C_1-C_{10} alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, ocytylene, nonylene and decalene.

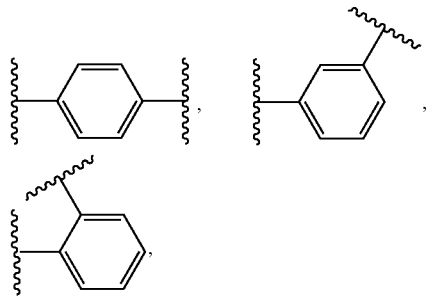
"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene (—CH═CH—).

"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene (—C≡C—), propargyl (—CH_2C≡C—), and 4-pentynyl (—$CH_2CH_2CH_2$C≡C—).

"Aryl" refers to a carbocyclic aromatic group. Examples of aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A carbocyclic aromatic group or a heterocyclic aromatic group can be unsubstituted or substituted with one or more groups including, but not limited to, —C_1-C_8 alkyl, —O—(C_1-C_8 alkyl), -aryl, —C(O)R', —OC(O)R', —C(O)OR, —$C(O)NH_2$, —C(O)NHR', —$C(O)N(R')_2$—NHC(O)R', —$S(O)_2$R', —S(O)R', —OH, -halogen, —N_3, —NH_2, —NH(R'), —$N(R')_2$ and —CN; wherein each R' is independently selected from H, —C_1-C_8 alkyl and aryl.

A "C_5-C_{20} aryl" is an aryl group with 5 to 20 carbon atoms in the carbocyclic aromatic rings. Examples of C_5-C_{20} aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A C_5-C_{20} aryl group can be substituted or unsubstituted as described above for aryl groups. A "C_5-C_{14} aryl" is an aryl group with 5 to 14 carbon atoms in the carbocyclic aromatic rings. Examples of C_5-C_{14} aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A C_5-C_{14} aryl group can be substituted or unsubstituted as described above for aryl groups.

An "arylene" is an aryl group which has two covalent bonds and can be in the ortho, meta, or para configurations as shown in the following structures:



in which the phenyl group can be unsubstituted or substituted with up to four groups including, but not limited to, —C_1-C_8 alkyl, —O—(C_1-C_8 alkyl), -aryl, —C(O)R', —OC(O)R', —C(O)OR, —$C(O)NH_2$, —C(O)NHR', —$C(O)N(R')_2$—NHC(O)R', —$S(O)_2$R', —S(O)R', —OH, -halogen, —N_3, —NH_2, —NH(R'), —$N(R')_2$ and —CN; wherein each R' is independently selected from H, —C_1-C_8 alkyl and aryl.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl radical. Typical heteroarylalkyl groups include, but are not limited to, 2-benzimidazolylmethyl, 2-furylethyl, and the like. The heteroarylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the heteroarylalkyl group is 1 to 6 carbon atoms and the heteroaryl moiety is 5 to 14 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. The heteroaryl moiety of the heteroarylalkyl group may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo[4,5], [5,5], [5,6], or [6,6] system.

"Substituted alkyl," "substituted aryl," and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, —X, —R, —O^-, —OR, —SR, —S^-, —NR_2, —NR_3, ═NR, —CX_3, —CN, —OCN, —SCN, —N═C═O, —NCS, —NO, —NO_2, ═N_2, —N_3, NC(═O)R, —C(═O)R, —$C(═O)NR_2$, —SO_3—, —SO_3H, —$S(═O)_2$R, —$OS(═O)_2$OR, —$S(═O)_2NR$, —S(═O)R, —$OP(═O)(OR)_2$, —$P(═O)(OR)_2$, —PO^-_3, —PO_3H_2, —C(═O)R, —C(═O)X, —C(═S)R, —CO_2R, —CO_2^-, —C(═S)OR, —C(═O)SR, —C(═S)SR, —$C(═O)NR_2$, —$C(═S)NR_2$, —$C(═NR)NR_2$, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently —H, C_2-C_{18} alkyl, C_6-C_{20} aryl, C_3-C_{14} heterocycle, protect-

ing group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups as described above may also be similarly substituted.

"Heteroaryl" and "heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. The heterocycle radical comprises 3 to 20 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo[4,5], [5,5], [5,6], or [6,6] system.

Exemplary heterocycles are described, e.g., in Paquette, Leo A., "Principles of Modern Heterocyclic Chemistry" (W. A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.* (1960) 82:5566.

Examples of heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, and isatinoyl.

By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

A "C_3-C_8 heterocycle" refers to an aromatic or non-aromatic C_3-C_8 carbocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. Representative examples of a C_3-C_8 heterocycle include, but are not limited to, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl, pyrrolyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl and tetrazolyl. A C_3-C_8 heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, —C_1-C_8 alkyl, —O—(C_1-C_8 alkyl), -aryl, —C(O)R', —OC(O)R', —C(O)OR', —$C(O)NH_2$, —C(O)NHR', —$C(O)N(R')_2$—NHC(O)R', —$S(O)_2R'$, —S(O)R', —OH, -halogen, —N_3, —NH_2, —NH(R'), —$N(R')_2$ and —CN; wherein each R' is independently selected from H, —C_1-C_8 alkyl and aryl.

"C_3-C_8 heterocyclo" refers to a C_3-C_8 heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond. A C_3-C_8 heterocyclo can be unsubstituted or substituted with up to six groups including, but not limited to, —C_1-C_8 alkyl, —O—(C_1-C_8 alkyl), -aryl, —C(O)R', —OC(O)R', —C(O)OR, —$C(O)NH_2$, —C(O)NHR', —$C(O)N(R')_2$—NHC(O)R', —$S(O)_2R'$, —S(O)R', —OH, -halogen, —N_3, —NH_2, —NH(R'), —$N(R')_2$ and —CN; wherein each R' is independently selected from H, —C_1-C_8 alkyl and aryl.

A "C_3-C_{20} heterocycle" refers to an aromatic or non-aromatic C_3-C_8 carbocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. A C_3-C_{20} heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, —C_1-C_8 alkyl, —O—(C_1-C_8 alkyl), -aryl, —C(O)R', —OC(O)R', —C(O)OR, —$C(O)NH_2$, —C(O)NHR', —$C(O)N(R')_2$—NHC(O)R', —$S(O)_2R'$, —S(O)R', —OH, -halogen, —N_3, —NH_2, —NH(R'), —$N(R')_2$ and —CN; wherein each R' is independently selected from H, —C_1-C_8 alkyl and aryl.

"C_3-C_{20} heterocyclo" refers to a C_3-C_{20} heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond.

"Carbocycle" means a saturated or unsaturated ring having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, e.g. arranged as a bicyclo[4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo[5,6] or [6,6] system. Examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cycloheptyl, and cyclooctyl.

A "C_3-C_8 carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C_3-C_8 carbocycles include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexenyl, -1,3-cyclohexadienyl, -1,4-cyclohexadienyl, -cycloheptyl, -1,3-cycloheptadienyl, -1,3,5-cycloheptatrienyl, -cyclooctyl, and -cyclooctadienyl. A C_3-C_8 carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, —C_1-C_8 alkyl, —O—(C_1-C_8 alkyl), -aryl, —C(O)R', —OC(O)R', —C(O)OR, —$C(O)NH_2$, —C(O)NHR', —$C(O)N(R')_2$—NHC(O)R', —$S(O)_2R'$, —S(O)R', —OH, -halogen, —N_3,

—NH_2, —NH(R'), —$N(R')_2$ and —CN; where each R' is independently selected from H, —C_1-C_8 alkyl and aryl.

A "C_3-C_8 carbocyclo" refers to a C_3-C_8 carbocycle group defined above wherein one of the carbocycle groups' hydrogen atoms is replaced with a bond.

"Linker" refers to a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. In various embodiments, linkers include a divalent radical such as an alkyldiyl, an aryldiyl, a heteroaryldiyl, moieties such as: —$(CR_2)_nO(CR_2)_n$—, repeating units of alkyloxy (e.g. polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (e.g. polyethyleneamino, Jeffamine™); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide. In various embodiments, linkers can comprise one or more amino acid residues, such as valine, phenylalanine, lysine, and homolysine.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (−) are employed to designate the sign of rotation of plane-polarized light by the compound, with (−) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

"Leaving group" refers to a functional group that can be substituted by another functional group. Certain leaving groups are well known in the art, and examples include, but are not limited to, a halide (e.g., chloride, bromide, iodide), methanesulfonyl (mesyl), p-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), and trifluoromethylsulfonate.

The term "protecting group" refers to a substituent that is commonly employed to block or protect a particular functionality while reacting other functional groups on the compound. For example, an "amino-protecting group" is a substituent attached to an amino group that blocks or protects the amino functionality in the compound. Suitable amino-protecting groups include, but are not limited to, acetyl, trifluoroacetyl, t-butoxycarbonyl (BOC), benzyloxycarbonyl (CBZ) and 9-fluorenylmethylenoxycarbonyl (Fmoc). For a general description of protecting groups and their use, see T. W. Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, New York, 1991, or a later edition.

II. Compositions and Methods

In one aspect, the invention is based, in part, on antibodies that bind to PMEL17 and immunoconjugates comprising such antibodies. Antibodies and immunoconjugates of the invention are useful, e.g., for the diagnosis or treatment of PMEL17-positive cancers.

A. Exemplary Anti-PMEL17 Antibodies

In some embodiments, the invention provides isolated antibodies that bind to PMEL17. PMEL17 is an integral membrane protein the is presented transiently at the cell surface of melanocytes. As demonstrated herein, PMEL17 is expressed in the majority of human melanoma specimens examined.

An exemplary naturally occurring human PMEL17 preproprotein sequence, with signal sequence (amino acids 1-24) is provided in SEQ ID NO: 26, and the corresponding PMEL17 proprotein sequence is shown in SEQ ID NO: 27 (corresponding to amino acids 25-661 of SEQ ID NO: 26).

In certain embodiments, an anti-PMEL17 antibody binds an epitope within amino acids 105 to 125 of SEQ ID NO: 26, or binds to an epitope within amino acids 25 to 45 of SEQ ID NO: 26. Nonlimiting exemplary such antibodies include 17A9, 8G3, and 31D1. In some embodiments, an anti-PMEL17 antibody binds human PMEL17. In some embodiments, an anti-PMEL17 antibody binds PMEL selected from human, cynomolgus monkey, mouse, and rat PMEL17.

In some embodiments, an anti-PMEL17 antibody binds to an epitope within amino acids 105 to 125 of SEQ ID NO: 26. In some such embodiments, the anti-PMEL17 antibody binds PMEL17 with an affinity of ≤5 nM, or ≤4 nM, or ≤3 nM, or ≤2 nM, or ≤1 nM, and optionally ≥0.0001 nM, or ≥0.001 nM, or ≥0.01 nM. Nonlimiting exemplary such antibodies include 17A9 and 8G3, described herein. In some embodiments, an anti-PMEL17 antibody that binds to an epitope within amino acids 105 to 125 of SEQ ID NO: 26 binds to human PMEL17. In some embodiments, an anti-PMEL17 antibody that binds to an epitope within amino acids 105 to 125 of SEQ ID NO: 26 binds to human PMEL17 and/or cynomolgus monkey PMEL17.

In some embodiments, an anti-PMEL17 antibody binds to an epitope within amino acids 25 to 45 of SEQ ID NO: 26. In some such embodiments, the anti-PMEL17 antibody binds PMEL17 with an affinity of ≤5 nM, or ≤4 nM, or ≤3 nM, or ≤2 nM, or ≤1 nM, and optionally ≥0.0001 nM, or ≥0.001 nM, or ≥0.01 nM. A nonlimiting exemplary such antibody is 31D1, described herein. In some embodiments, an anti-PMEL17 antibody that binds to an epitope within amino acids 25 to 45 of SEQ ID NO: 26 binds to human PMEL17. In some embodiments, an anti-PMEL17 antibody that binds to an epitope within amino acids 105 to 125 of SEQ ID NO: 26 binds to human PMEL17 and/or cynomolgus monkey PMEL17.

Further antibodies that bind PMEL17 are provided. A nonlimiting exemplary anti-PMEL17 antibody is 15F2, described herein. In some embodiments, the anti-PMEL17 antibody binds PMEL17 with an affinity of ≤5 nM, or ≤4 nM, or ≤3 nM, or ≤2 nM, or ≤1 nM, and optionally ≥0.0001 nM, or ≥0.001 nM, or ≥0.01 nM. In some embodiments, an anti-

PMEL17 antibody binds to human PMEL17. In some embodiments, an anti-PMEL17 antibody binds to human PMEL17 and/or cynomolgus monkey PMEL17.

Assays

To determine whether an anti-PMEL17 antibody "binds to an epitope within amino acids 105 to 125 of SEQ ID NO: 26" or "binds to an epitope within amino acids 25 to 45 of SEQ ID NO: 26," PMEL17 polypeptides with N- and C-terminal deletions are expressed in 293 cells and binding of the antibody to the truncated polypeptides is tested by FACS as described in Example D, wherein a substantial reduction (≥70% reduction) or elimination of binding of the antibody to a truncated polypeptide relative to binding to full-length PMEL17 expressed in 293 cells indicates that the antibody does not bind to that truncated polypeptide.

Whether an anti-PMEL17 antibody "binds with an affinity of ≤5 nM, or ≤4 nM, or ≤3 nM, or ≤2 nM, or ≤1 nM," is determined according to a BIAcore® assay. For example, Kd is measured using surface plasmon resonance assays using a BIACORE®-3000 (BIAcore, Inc., Piscataway, N.J.). BIAcore™ research grade CM5 chips are activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) reagents according to the supplier's instructions. Goat anti-human Fc IgGs are coupled to the chips to achieve approximately 10,000 response units (RU) in each flow cell. Unreacted coupling groups are blocked with 1M ethanolamine. For kinetics measurements, anti-PMEL17 antibodies are captured to achieve approximately 300 RU. Two-fold serial dilutions of human PMEL17 ECD (for example, amino acids 25 to 291 fused to a C-terminal Fc expressed in a baculovirus system, or amino acids 22-558 fused to Fc; 125 nM to 0.49 nM) are injected in HBS-P buffer (0.01M HEPES pH7.4, 0.15M NaCl, 0.005% surfactant P20) at 25° C. with a flow rate of 30 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a 1:1 Langmuir binding model (BIAcore™ Evaluation Software version 3.2). The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass) at 25° C. of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-Aminco® spectrophotometer (ThermoSpectronic) with a stirred cuvette.

Antibody 17A9 and Other Embodiments

In some embodiments, the invention provides an anti-PMEL17 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 5; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In some embodiments, the invention provides an anti-PMEL17 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID

NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 5; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In some embodiments, X_1 in SEQ ID NO: 17 is selected from Arg, Lys, Gln, Asn, Gly, and Ala. In some embodiments, X_1 in SEQ ID NO: 17 is selected from Arg, Lys, Gly, and Ala. In some embodiments, X_1 in SEQ ID NO: 17 is selected from Arg and Gly. In some embodiments, X_2 in SEQ ID NO: 18 is selected from Tyr, Trp, Phe, Thr, Ser, Ile, Leu, Val, Met, Ala, and Norleucine. In some embodiments, X_2 in SEQ ID NO: 18 is selected from Tyr, Phe, Ile, Leu, and Val. In some embodiments, X_2 in SEQ ID NO: 18 is selected from Tyr and Ile. In some embodiments, X_3 in SEQ ID NO: 19 is selected from Ser, Thr, and Val. In some embodiments, X_3 in SEQ ID NO: 19 is selected from Ser and Thr. In some embodiments, X_4 in SEQ ID NO: 19 is selected from Ser, Thr, and Val. In some embodiments, X_4 in SEQ ID NO: 19 is selected from Ser and Thr.

In any of the above embodiments, an anti-PMEL17 antibody is humanized. In one embodiment, an anti-PMEL17 antibody comprises HVRs as in any of the above embodiments, and further comprises a human acceptor framework, e.g. a human immunoglobulin framework or a human consensus framework. In certain embodiments, the human acceptor framework is the human VL kappa 1 (VL_{KI}) framework and/or the VH framework VH_1. In some embodiments, a humanized anti-PMEL17 antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, a humanized anti-PMEL17 antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, an anti-PMEL17 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 1 or 50. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 1 or 50 contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PMEL17 antibody comprising that sequence retains the ability to bind to PMEL17. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 1 or 50. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 1 or 50. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PMEL17 antibody comprises the VH sequence of SEQ ID NO: 1 or 50, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5. In some embodiments, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5.

In some embodiments, an anti-PMEL17 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 2 contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PMEL17 antibody comprising that sequence retains the ability to bind to PMEL17. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 2. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 2. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PMEL17 antibody comprises the VL sequence of SEQ ID NO: 2, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (b)

HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, an anti-PMEL17 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 1 and SEQ ID NO: 2, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 50 and SEQ ID NO: 2, respectively, including post-translational modifications of those sequences.

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-PMEL17 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-PMEL17 antibody comprising a VH sequence of SEQ ID NO: 1 or 50 and a VL sequence of SEQ ID NO: 2. In certain embodiments, an antibody is provided that binds to an epitope of SEQ ID NO: 26 from, within, or overlapping amino acids 105 to 125.

In a further aspect of the invention, an anti-PMEL17 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-PMEL17 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a substantially full length antibody, e.g., an IgG1 antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-PMEL17 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

Antibody 8G3 and Other Embodiments

In some embodiments, the invention provides an anti-PMEL17 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 15; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In some embodiments, the invention provides an anti-PMEL17 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 15; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In some embodiments, X_1 in SEQ ID NO: 17 is selected from Arg, Lys, Gln, Asn, Gly, and Ala. In some embodiments, X_1 in SEQ ID NO: 17 is selected from Arg, Lys, Gly, and Ala. In some embodiments, X_1 in SEQ ID NO: 17 is selected from Arg and Gly. In some embodiments, X_2 in SEQ ID NO: 18 is selected from Tyr, Trp, Phe, Thr, Ser, Ile, Leu, Val, Met, Ala, and Norleucine. In some embodiments, X_2 in SEQ ID NO: 18 is selected from Tyr, Phe, Ile, Leu, and Val. In some embodiments, X_2 in SEQ ID NO: 18 is selected from Tyr and Ile. In some embodiments, X_3 in SEQ ID NO: 19 is selected from Ser, Thr, and Val. In some embodiments, X_3 in SEQ ID NO: 19 is selected from Ser and Thr. In some embodiments, X_4 in SEQ ID NO: 19 is selected from Ser, Thr, and Val. In some embodiments, X_4 in SEQ ID NO: 19 is selected from Ser and Thr.

In any of the above embodiments, an anti-PMEL17 antibody is humanized. In one embodiment, an anti-PMEL17 antibody comprises HVRs as in any of the above embodiments, and further comprises a human acceptor framework, e.g. a human immunoglobulin framework or a human consensus framework. In some embodiments, a humanized anti-PMEL17 antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, a humanized anti-PMEL17 antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, an anti-PMEL17 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 9 or 49. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 9 or 49 contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PMEL17 antibody comprising that sequence retains the ability to bind to PMEL17. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 9 or 49. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 9 or 49. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PMEL17 antibody comprises the VH sequence of SEQ ID NO: 9 or 49, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 17, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15.

In some embodiments, an anti-PMEL17 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 10. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 10 contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PMEL17 antibody comprising that sequence retains the ability to bind to PMEL17. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 10. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 10. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PMEL17 antibody comprises the VL sequence of SEQ ID NO: 10, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 19; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, an anti-PMEL17 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 9 and SEQ ID NO: 10, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 49 and SEQ ID NO: 10, respectively, including post-translational modifications of those sequences.

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-PMEL17 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-PMEL17 antibody comprising a VH sequence of SEQ ID

NO: 9 or 49 and a VL sequence of SEQ ID NO: 10. In certain embodiments, an antibody is provided that binds to an epitope of SEQ ID NO: 26 from, within, or overlapping amino acids 105 to 125.

In a further aspect of the invention, an anti-PMEL17 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-PMEL17 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment, the antibody is a substantially full length antibody, e.g., an IgG1 antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-PMEL17 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

Antibody 15F2 and Other Embodiments

In some embodiments, the invention provides an anti-PMEL17 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22. In one embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 22; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25.

In any of the above embodiments, an anti-PMEL17 antibody is humanized. In one embodiment, an anti-PMEL17 antibody comprises HVRs as in any of the above embodiments, and further comprises a human acceptor framework, e.g. a human immunoglobulin framework or a human consensus framework. In some embodiments, a humanized anti-PMEL17 antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25.

In another aspect, an anti-PMEL17 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 11 or 51. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 11 or 51 contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PMEL17 antibody comprising that sequence retains the ability to bind to PMEL17. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 11 or 51. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 11 or 51. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PMEL17 antibody comprises the VH sequence of SEQ ID NO: 11 or 51, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22.

In some embodiments, an anti-PMEL17 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 12. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 12 contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PMEL17 antibody comprising that sequence retains the ability to bind to PMEL17. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 12. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 12. In certain embodiments, the substitutions, insertions, or dele-

tions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PMEL17 antibody comprises the VL sequence of SEQ ID NO: 12, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25.

In another aspect, an anti-PMEL17 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO: 11 and SEQ ID NO: 12, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 51 and SEQ ID NO: 12, respectively, including post-translational modifications of those sequences.

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-PMEL17 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-PMEL17 antibody comprising a VH sequence of SEQ ID NO: 11 or 51 and a VL sequence of SEQ ID NO: 12.

In a further aspect of the invention, an anti-PMEL17 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-PMEL17 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment, the antibody is a substantially full length antibody, e.g., an IgG1 antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-PMEL17 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

Antibody 31D1 and Other Embodiments

In one aspect, the invention provides an anti-PMEL17 antibody comprising at least one, two, three, four, five, or six HVRs of the antibody produced by the hybridoma designated "7509(31D1.6.7)" and deposited with the ATCC on Apr. 24, 2012 and having ATCC Accession No. PTA-12862. For purposes of this section, HVRs are delineated by the amino acid ranges corresponding to CDRs, i.e., CDRs that occur at amino acid residues 24-34, 50-56, and 89-97 of the light chain variable region, and amino acid residues 31-35B, 50-65, and 95-102 of the heavy chain variable region (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3, respectively). See Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991).

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In one embodiment, the antibody comprises HVR-H3 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In another embodiment, the antibody comprises HVR-H3 and HVR-L3 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In a further embodiment, the antibody comprises HVR-H3, HVR-L3, and HVR-H2 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In a further embodiment, the antibody comprises HVR-H1, HVR-H2, and HVR-H3 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In one embodiment, the antibody comprises HVR-L1, HVR-L2, and HVR-L3 of the antibody produced by hybridoma 7509 (31D1.6.7) having ATCC Accession No. PTA-12862.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In another aspect, the invention provides an antibody comprising HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 of the antibody produced by hybridoma 7509 (31D1.6.7) having ATCC Accession No. PTA-12862.

In any of the above embodiments, an anti-PMEL17 antibody is humanized. In one such embodiment, the antibody is a humanized form of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In a further embodiment, an anti-PMEL17 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework.

In another aspect, an anti-PMEL17 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the VH of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In certain embodiments, a VH sequence contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PMEL17 antibody comprising that sequence retains the ability to bind to PMEL17. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the VH of the antibody produced by hybridoma 7509 (31D1.6.7) having ATCC Accession No. PTA-12862. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PMEL17 antibody comprises the VH sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two, or three HVRs selected from HVR-H1, HVR-H2, and HVR-H3 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In another aspect, an anti-PMEL17 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the VL of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PMEL17 antibody comprising that sequence retains the ability to bind to PMEL17. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the VL of the antibody produced by hybridoma 7509(31D1.6.7) hav-

ing ATCC Accession No. PTA-12862. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-PMEL17 antibody comprises the VL sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from HVR-L1, HVR-L2, and HVR-L3 of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In another aspect, an anti-PMEL17 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862, respectively, including post-translational modifications of those sequences.

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-PMEL17 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

In a further aspect of the invention, an anti-PMEL17 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-PMEL17 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment, the antibody is a substantially full length antibody, e.g., an IgG2b antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-PMEL17 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of ≤1 μM, ≤100 nM, ≤10 nM, ≤1 nM, ≤0.1 nM, ≤0.01 nM, or ≤0.001 nM, and optionally is $\geq 10^{-13}$ M. (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23° C.). In a non-adsorbent plate (Nunc #269620), 100 μM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, N.J.) at 25° C. with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE®, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 l/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25° C. at a flow rate of approximately 25 l/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 106 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass) at 25° C. of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthiin, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Pat. Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Pat. No. 5,869,046.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, Mass.; see, e.g., U.S. Pat. No. 6,248,516 B1).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA,* 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall' Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer,* 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA,* 89:4285 (1992); and Presta et al. *J. Immunol.,* 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

In certain embodiments, an antibody provided herein is a human antibody.

Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Pat. Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Pat. No. 5,770,429 describing HuMAb® technology; U.S. Pat. No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VelociMouse® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.,* 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.,* 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA,* 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Pat. No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue,* 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology,* 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology,* 27(3): 185-91 (2005).

Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, N.J., 2001) and further described, e.g., in the McCafferty et al., *Nature* 348: 552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, N.J., 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101 (34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132 (2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which

can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.,* 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter, *J. Mol. Biol.,* 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for PMEL17 and the other is for any other antigen. In certain embodiments, one of the binding specificities is for PMEL17 and the other is for CD3. See, e.g., U.S. Pat. No. 5,821,337. In certain embodiments, bispecific antibodies may bind to two different epitopes of PMEL17. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express PMEL17. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Pat. No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., U.S. Pat. No. 4,676,980, and Brennan et al., *Science,* 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.,* 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA,* 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,e.g. Gruber et al., *J. Immunol.,* 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to PMEL17 as well as another, different antigen (see, US 2008/0069820, for example).

7. Antibody Variants

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
(3) acidic: Asp, Glu;
(4) basic: H is, Lys, Arg;
(5) residues that influence chain orientation: Gly, Pro;
(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained

certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, N.J., (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science,* 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex is used to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation Variants

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH_2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ±3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec 13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.,* 94(4):680-688 (2006); and WO2003/085107).

Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); U.S. Pat. No. 6,602,684 (Umana et al.);

and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc Region Variants

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'lAcad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166: 1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). Clq binding assays may also be carried out to confirm that the antibody is unable to bind Clq and hence lacks CDC activity. See, e.g., Clq and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg, M. S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S. B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826).

See also Duncan & Winter, Nature 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine Engineered Antibody Variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Pat. No. 7,521,541.

e) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone) polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are

attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Pat. No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-PMEL17 antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-PMEL17 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of an anti-PMEL17 antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Pat. Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology*, Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures can also be utilized as hosts. See, e.g., U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383: 44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J.), pp. 255-268 (2003).

C. Assays

Anti-PMEL17 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, BIACore®, FACS, or Western blot.

In another aspect, competition assays may be used to identify an antibody that competes with any of the antibodies described herein for binding to PMEL17. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by an antibody described herein. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, N.J.).

In an exemplary competition assay, immobilized PMEL17 is incubated in a solution comprising a first labeled antibody that binds to PMEL17 (e.g., any of the antibodies described herein) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to PMEL17. The second antibody may be present in a hybridoma supernatant. As a control, immobilized PMEL17 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to PMEL17, excess unbound antibody is removed, and the

amount of label associated with immobilized PMEL17 is measured. If the amount of label associated with immobilized PMEL17 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to PMEL17. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch. 14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

D. Immunoconjugates

The invention also provides immunoconjugates comprising an anti-PMEL17 antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes (i.e., a radioconjugate).

Immunoconjugates allow for the targeted delivery of a drug moiety to a tumor, and, in some embodiments intracellular accumulation therein, where systemic administration of unconjugated drugs may result in unacceptable levels of toxicity to normal cells (Polakis P. (2005) *Current Opinion in Pharmacology* 5:382-387).

Antibody-drug conjugates (ADC) are targeted chemotherapeutic molecules which combine properties of both antibodies and cytotoxic drugs by targeting potent cytotoxic drugs to antigen-expressing tumor cells (Teicher, B. A. (2009) *Current Cancer Drug Targets* 9:982-1004), thereby enhancing the therapeutic index by maximizing efficacy and minimizing off-target toxicity (Carter, P. J. and Senter P. D. (2008) *The Cancer Jour.* 14(3):154-169; Chari, R. V. (2008) *Acc. Chem. Res.* 41:98-107.

The ADC compounds of the invention include those with anticancer activity. In some embodiments, the ADC compounds include an antibody conjugated, i.e. covalently attached, to the drug moiety. In some embodiments, the antibody is covalently attached to the drug moiety through a linker. The antibody-drug conjugates (ADC) of the invention selectively deliver an effective dose of a drug to tumor tissue whereby greater selectivity, i.e. a lower efficacious dose, may be achieved while increasing the therapeutic index ("therapeutic window").

The drug moiety (D) of the antibody-drug conjugates (ADC) may include any compound, moiety or group that has a cytotoxic or cytostatic effect. Drug moieties may impart their cytotoxic and cytostatic effects by mechanisms including but not limited to tubulin binding, DNA binding or intercalation, and inhibition of RNA polymerase, protein synthesis, and/or topoisomerase. Exemplary drug moieties include, but are not limited to, a maytansinoid, dolastatin, auristatin, calicheamicin, pyrrolobenzodiazepine (PBD), nemorubicin and its derivatives, PNU-159682, anthracycline, duocarmycin, vinca alkaloid, taxane, trichothecene, CC1065, camptothecin, elinafide, and stereoisomers, isosteres, analogs, and derivatives thereof that have cytotoxic activity. Nonlimiting examples of such immunoconjugates are discussed in further detail below.

1. Exemplary Antibody-Drug Conjugates

An exemplary embodiment of an antibody-drug conjugate (ADC) compound comprises an antibody (Ab) which targets a tumor cell, a drug moiety (D), and a linker moiety (L) that attaches Ab to D. In some embodiments, the antibody is attached to the linker moiety (L) through one or more amino acid residues, such as lysine and/or cysteine.

An exemplary ADC has Formula I:

$$Ab\text{-}(L\text{-}D)_p \qquad I$$

where p is 1 to about 20. In some embodiments, the number of drug moieties that can be conjugated to an antibody is limited by the number of free cysteine residues. In some embodiments, free cysteine residues are introduced into the antibody amino acid sequence by the methods described herein. Exemplary ADC of Formula I include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon, R. et al (2012) *Methods in Enzym.* 502:123-138). In some embodiments, one or more free cysteine residues are already present in an antibody, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the antibody to a drug. In some embodiments, an antibody is exposed to reducing conditions prior to conjugation of the antibody in order to generate one or more free cysteine residues.

a) Exemplary Linkers

A "Linker" (L) is a bifunctional or multifunctional moiety that can be used to link one or more drug moieties (D) to an antibody (Ab) to form an antibody-drug conjugate (ADC) of Formula I. In some embodiments, antibody-drug conjugates (ADC) can be prepared using a Linker having reactive functionalities for covalently attaching to the drug and to the antibody. For example, in some embodiments, a cysteine thiol of an antibody (Ab) can form a bond with a reactive functional group of a linker or a drug-linker intermediate to make an ADC.

In one aspect, a linker has a functionality that is capable of reacting with a free cysteine present on an antibody to form a covalent bond. Nonlimiting exemplary such reactive functionalities include maleimide, haloacetamides, α-haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates, and isothiocyanates. See, e.g., the conjugation method at page 766 of Klussman, et al (2004), *Bioconjugate Chemistry* 15(4):765-773, and the Examples herein.

In some embodiments, a linker has a functionality that is capable of reacting with an electrophilic group present on an antibody. Exemplary such electrophilic groups include, but are not limited to, aldehyde and ketone carbonyl groups. In some embodiments, a heteroatom of the reactive functionality of the linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Nonlimiting exemplary such reactive functionalities include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl (a "PAB"), N-Succinimidyl 4-(2-pyridylthio)pentanoate ("SPP"), and 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("MCC"). Various linker components are known in the art, some of which are described below.

A linker may be a "cleavable linker," facilitating release of a drug. Nonlimiting exemplary cleavable linkers include acid-labile linkers (e.g., comprising hydrazone), protease-sensitive (e.g., peptidase-sensitive) linkers, photolabile linkers, or disulfide-containing linkers (Chari et al., Cancer Research 52:127-131 (1992); U.S. Pat. No. 5,208,020).

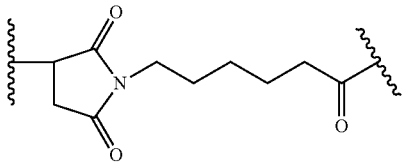
In certain embodiments, a linker has the following Formula II:

$$\text{-}A_a\text{-}W_w\text{—}Y_y\text{—} \qquad II$$

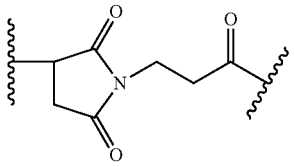
wherein A is a "stretcher unit", and a is an integer from 0 to 1; W is an "amino acid unit", and w is an integer from 0 to 12;

Y is a "spacer unit", and y is 0, 1, or 2; and Ab, D, and p are defined as above for Formula I. Exemplary embodiments of such linkers are described in U.S. Pat. No. 7,498,298, which is expressly incorporated herein by reference.

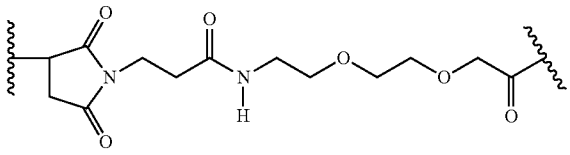
In some embodiments, a linker component comprises a "stretcher unit" that links an antibody to another linker component or to a drug moiety. Nonlimiting exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody, drug, or additional linker components):



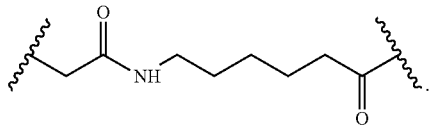
MC



MP



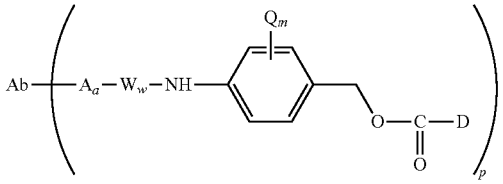
mPEG



In some embodiments, a linker component comprises an "amino acid unit". In some such embodiments, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular proteases, such as lysosomal enzymes (Doronina et al. (2003) *Nat. Biotechnol.* 21:778-784). Exemplary amino acid units include, but are not limited to, dipeptides, tripeptides, tetrapeptides, and pentapeptides. Exemplary dipeptides include, but are not limited to, valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe); phenylalanine-lysine (fk or phe-lys); phenylalanine-homolysine (phe-homolys); and N-methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include, but are not limited to, glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). An amino acid unit may comprise amino acid residues that occur naturally and/or minor amino acids and/or non-naturally occurring amino acid analogs, such as citrulline. Amino acid units can be designed and optimized for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

In some embodiments, a linker component comprises a "spacer" unit that links the antibody to a drug moiety, either directly or through a stretcher unit and/or an amino acid unit. A spacer unit may be "self-immolative" or a "non-self-immolative." A "non-self-immolative" spacer unit is one in which part or all of the spacer unit remains bound to the drug moiety upon cleavage of the ADC. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. In some embodiments, enzymatic cleavage of an ADC containing a glycine-glycine spacer unit by a tumor-cell associated protease results in release of a glycine-glycine-drug moiety from the remainder of the ADC. In some such embodiments, the glycine-glycine-drug moiety is subjected to a hydrolysis step in the tumor cell, thus cleaving the glycine-glycine spacer unit from the drug moiety.

A "self-immolative" spacer unit allows for release of the drug moiety. In certain embodiments, a spacer unit of a linker comprises a p-aminobenzyl unit. In some such embodiments, a p-aminobenzyl alcohol is attached to an amino acid unit via an amide bond, and a carbamate, methylcarbamate, or carbonate is made between the benzyl alcohol and the drug (Hamann et al. (2005) *Expert Opin. Ther. Patents* (2005) 15:1087-1103). In some embodiments, the spacer unit is p-aminobenzyloxycarbonyl (PAB). In some embodiments, an ADC comprising a self-immolative linker has the structure:

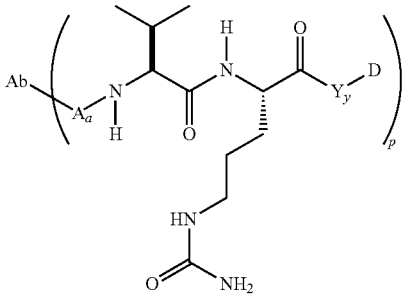


wherein Q is —C_1-C_8 alkyl, —O—(C_1-C_8 alkyl), -halogen, -nitro, or -cyano; m is an integer ranging from 0 to 4; and p ranges from 1 to about 20. In some embodiments, p ranges from 1 to 10, 1 to 7, 1 to 5, or 1 to 4.

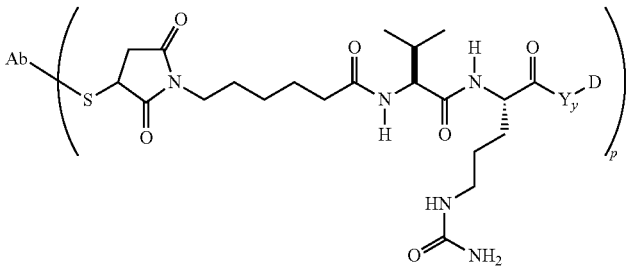
Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group, such as 2-aminoimidazol-5-methanol derivatives (U.S. Pat. No. 7,375,078; Hay et al. (1999) *Bioorg. Med. Chem. Lett.* 9:2237) and ortho- or para-aminobenzylacetals. In some embodiments, spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al (1995) *Chemistry Biology* 2:223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm et al (1972) *J. Amer. Chem. Soc.* 94:5815) and 2-aminophenylpropionic acid amides (Amsberry, et al (1990) *J. Org. Chem.* 55:5867). Linkage of a drug to the α-carbon of a glycine residue is another example of a self-immolative spacer that may be useful in ADC (Kingsbury et al (1984) *J. Med. Chem.* 27:1447).

In some embodiments, linker L may be a dendritic type linker for covalent attachment of more than one drug moiety to an antibody through a branching, multifunctional linker moiety (Sun et al (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215; Sun et al (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768). Dendritic linkers can increase the molar ratio of drug to antibody, i.e. loading, which is related to the potency of the ADC. Thus, where an antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

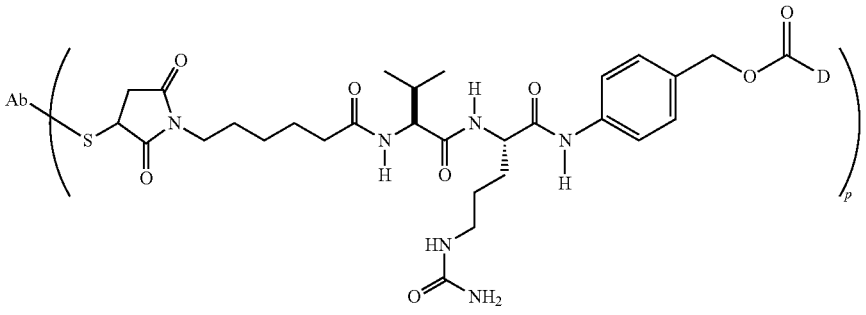
Nonlimiting exemplary linkers are shown below in the context of an ADC of Formula I:



val-cit

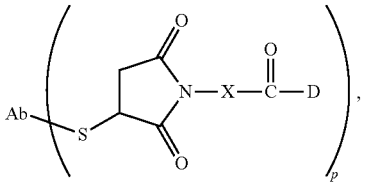


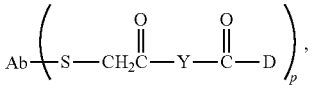
MC-val-cit

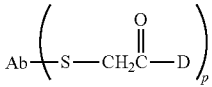


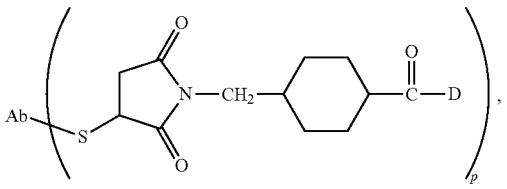
MC-val-cit-PAB

Further nonlimiting exemplary ADCs include the structures:









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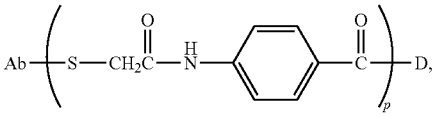
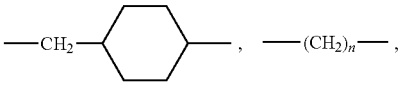
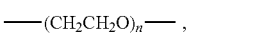
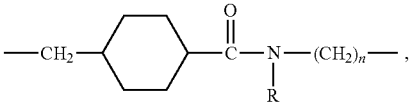
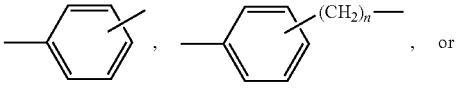


where X is:









or

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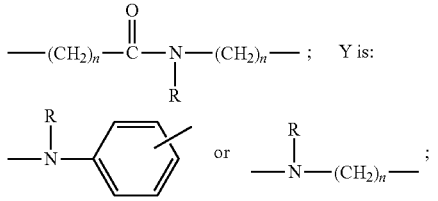
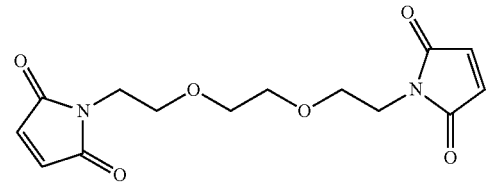


each R is independently H or C_1-C_6 alkyl; and n is 1 to 12.

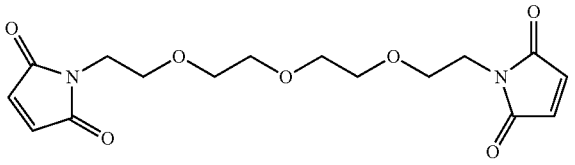
Typically, peptide-type linkers can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to a liquid phase synthesis method (e.g., E. Schröder and K. Lübke (1965) "The Peptides", volume 1, pp 76-136, Academic Press).

In some embodiments, a linker is substituted with groups that modulate solubility and/or reactivity. As a nonlimiting example, a charged substituent such as sulfonate ($—SO_3^-$) or ammonium may increase water solubility of the linker reagent and facilitate the coupling reaction of the linker reagent with the antibody and/or the drug moiety, or facilitate the coupling reaction of Ab-L (antibody-linker intermediate) with D, or D-L (drug-linker intermediate) with Ab, depending on the synthetic route employed to prepare the ADC. In some embodiments, a portion of the linker is coupled to the antibody and a portion of the linker is coupled to the drug, and then the Ab-(linker portion)^a is coupled to drug-(linker portion)^b to form the ADC of Formula I. In some such embodiments, the antibody comprises more than one (linker portion) a substituents, such that more than one drug is coupled to the antibody in the ADC of Formula I.

The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with the following linker reagents: bis-maleimido-trioxyethylene glycol (BMPEO), N-(β-maleimidopropyloxy)-N-hydroxy succinimide ester (BMPS), N-(ϵ-maleimidocaproyloxy) succinimide ester (EMCS), N-[γ-maleimidobutyryloxy]succinimide ester (GMBS), 1,6-hexane-bis-vinylsulfone (HBVS), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), 4-(4-N-Maleimidophenyl)butyric acid hydrazide (MPBH), succinimidyl 3-(bromoacetamido) propionate (SBAP), succinimidyl iodoacetate (SIA), succinimidyl (4-iodoacetyl)aminobenzoate (SIAB), N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), succinimidyl 6-[(beta-maleimidopropionamido) hexanoate](SMPH), iminothiolane (IT), sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and succinimidyl-(4-vinylsulfone) benzoate (SVSB), and including bis-maleimide reagents: dithiobismaleimidoethane (DTME), 1,4-Bismaleimidobutane (BMB), 1,4 Bismaleimidyl-2,3-dihydroxybutane (BMDB), bismaleimidohexane (BMH), bismaleimidoethane (BMOE), $BM(PEG)_2$ (shown below), and $BM(PEG)_3$ (shown below); bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). In some embodiments, bismaleimide reagents allow the attachment of the thiol group of a cysteine in the antibody to a thiol-containing drug moiety, linker, or linker-drug intermediate. Other functional groups that are reactive with thiol groups include, but are not limited to, iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.



$BM(PEG)_2$



$BM(PEG)_3$

Certain useful linker reagents can be obtained from various commercial sources, such as Pierce Biotechnology, Inc. (Rockford, Ill.), Molecular Biosciences Inc. (Boulder, Colo.), or synthesized in accordance with procedures described in the art; for example, in Toki et al (2002) *J. Org. Chem.* 67:1866-1872; Dubowchik, et al. (1997) *Tetrahedron Letters,* 38:5257-60; Walker, M. A. (1995) *J. Org. Chem.* 60:5352-5355; Frisch et al (1996) *Bioconjugate Chem.* 7:180-186; U.S. Pat. No. 6,214,345; WO 02/088172; US 2003130189; US2003096743; WO 03/026577; WO 03/043583; and WO 04/032828.

Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, e.g., WO94/11026.

b) Exemplary Drug Moieties

(1) Maytansine and Maytansinoids

In some embodiments, an immunoconjugate comprises an antibody conjugated to one or more maytansinoid molecules. Maytansinoids are derivatives of maytansine, and are mitotic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinoids are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody-drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification or derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

Certain maytansinoids suitable for use as maytansinoid drug moieties are known in the art and can be isolated from

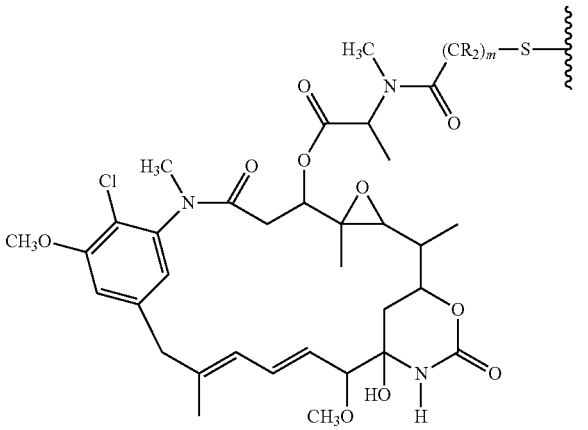
natural sources according to known methods or produced using genetic engineering techniques (see, e.g., Yu et al (2002) PNAS 99:7968-7973). Maytansinoids may also be prepared synthetically according to known methods.

Exemplary maytansinoid drug moieties include, but are not limited to, those having a modified aromatic ring, such as: C-19-dechloro (U.S. Pat. No. 4,256,746) (prepared, for example, by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl)+/−C-19-dechloro (U.S. Pat. Nos. 4,361,650 and 4,307,016) (prepared, for example, by demethylation using *Streptomyces* or *Actinomyces* or dechlorination using LAH); and C-20-demethoxy, C-20-acyloxy (—OCOR), +/−dechloro (U.S. Pat. No. 4,294,757) (prepared, for example, by acylation using acyl chlorides), and those having modifications at other positions of the aromatic ring.

Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (U.S. Pat. No. 4,424,219) (prepared, for example, by the reaction of maytansinol with H_2S or P_2S5); C-14-alkoxymethyl(demethoxy/CH_2OR) (U.S. Pat. No. 4,331,598); C-14-hydroxymethyl or acyloxymethyl (CH_2OH or CH_2OAc) (U.S. Pat. No. 4,450,254) (prepared, for example, from *Nocardia*); C-15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866) (prepared, for example, by the conversion of maytansinol by *Streptomyces*); C-15-methoxy (U.S. Pat. Nos. 4,313,946 and 4,315,929) (for example, isolated from Trewia nudlflora); C-18-N-demethyl (U.S. Pat. Nos. 4,362,663 and 4,322,348) (prepared, for example, by the demethylation of maytansinol by *Streptomyces*); and 4,5-deoxy (U.S. Pat. No. 4,371,533) (prepared, for example, by the titanium trichloride/LAH reduction of maytansinol).

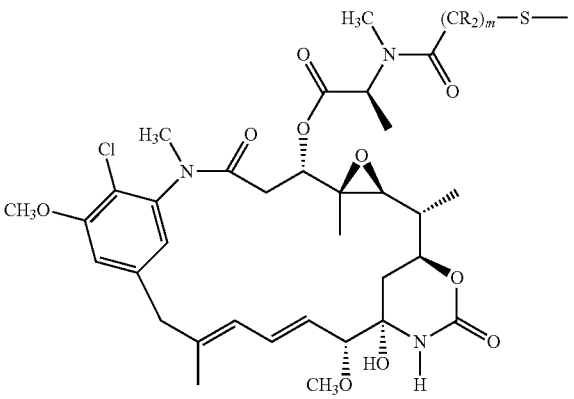
Many positions on maytansinoid compounds are useful as the linkage position. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. In some embodiments, the reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In some embodiments, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

Maytansinoid drug moieties include those having the structure:

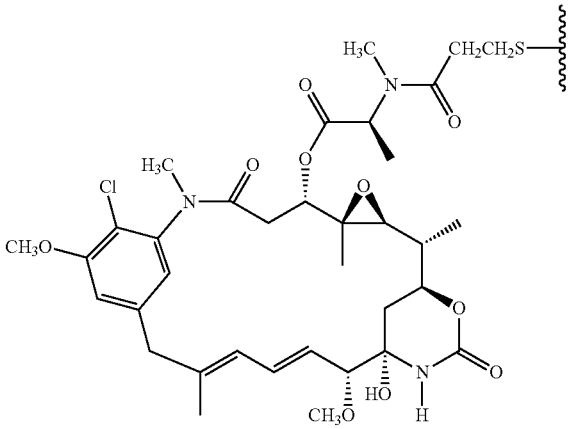


where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid drug moiety to a linker of an ADC. Each R may independently be H or a C_1-C_6 alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, i.e., m is 1, 2, or 3 (U.S. Pat. No. 633,410; U.S. Pat. No. 5,208,020; Chari et al (1992) *Cancer Res.* 52:127-131; Liu et al (1996) *Proc. Natl. Acad. Sci. USA* 93:8618-8623).

All stereoisomers of the maytansinoid drug moiety are contemplated for the ADC of the invention, i.e. any combination of R and S configurations at the chiral carbons (U.S. Pat. No. 7,276,497; U.S. Pat. No. 6,913,748; U.S. Pat. No. 6,441,163; U.S. Pat. No. 633,410 (RE39151); U.S. Pat. No. 5,208,020; Widdison et al (2006) J. Med. Chem. 49:4392-4408, which are incorporated by reference in their entirety). In some embodiments, the maytansinoid drug moiety has the following stereochemistry:

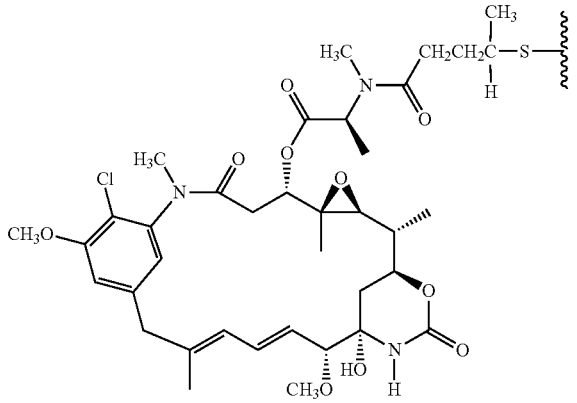


Exemplary embodiments of maytansinoid drug moieties include, but are not limited to, DM1; DM3; and DM4, having the structures:



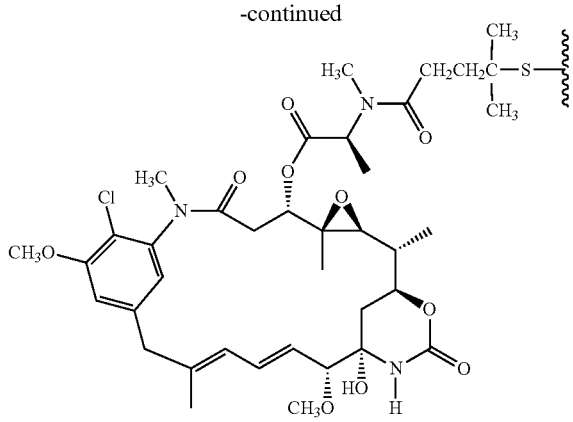
DM1

-continued



DM3

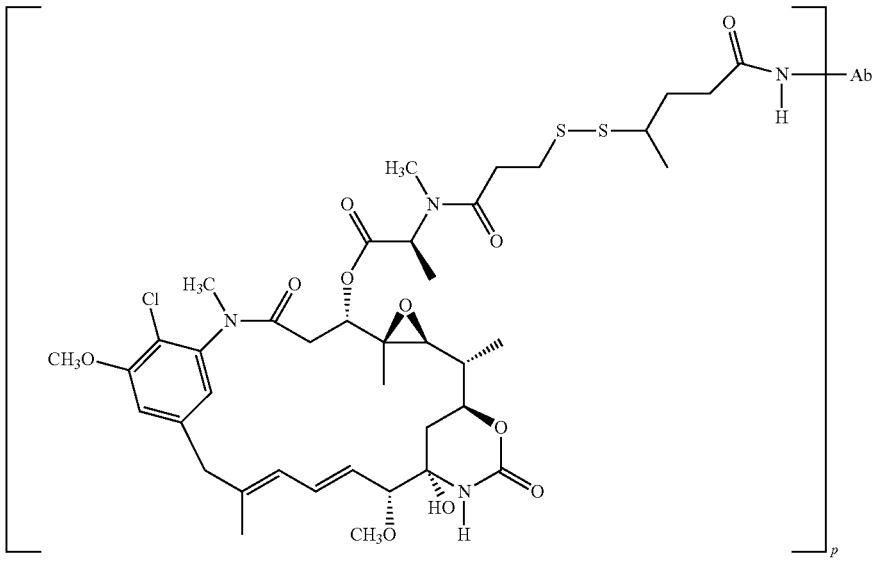
-continued



DM4

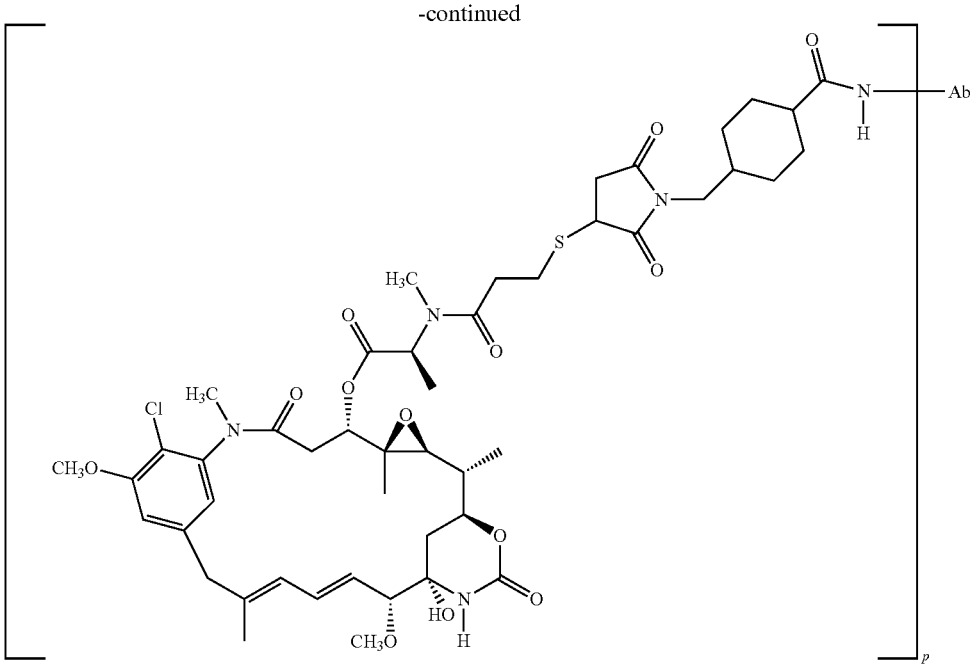
wherein the wavy line indicates the covalent attachment of the sulfur atom of the drug to a linker (L) of an antibody-drug conjugate.

Other exemplary maytansinoid antibody-drug conjugates have the following structures and abbreviations (wherein Ab is antibody and p is 1 to about 20. In some embodiments, p is 1 to 10, p is 1 to 7, p is 1 to 5, or p is 1 to 4):



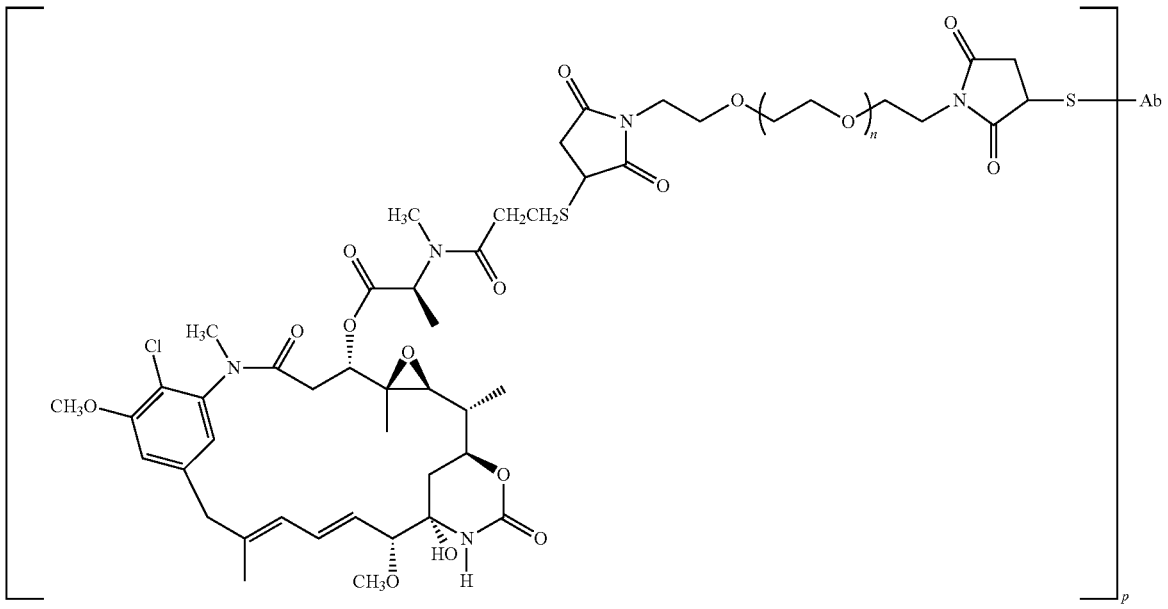
Ab-SPP-DM1

-continued



Ab-SMCC-DM1

Exemplary antibody-drug conjugates where DM1 is linked through a BMPEO linker to a thiol group of the antibody have the structure and abbreviation:



where Ab is antibody; n is 0, 1, or 2; and p is 1 to about 20. In some embodiments, p is 1 to 10, p is 1 to 7, p is 1 to 5, or p is 1 to 4.

Immunoconjugates containing maytansinoids, methods of making the same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020 and 5,416,064; US 2005/0276812 A1; and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. See also Liu et al. *Proc. Natl. Acad. Sci. USA* 93:8618-8623 (1996); and Chari et al. *Cancer Research* 52:127-131 (1992).

In some embodiments, antibody-maytansinoid conjugates may be prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the

biological activity of either the antibody or the maytansinoid molecule. See, e.g., U.S. Pat. No. 5,208,020 (the disclosure of which is hereby expressly incorporated by reference). In some embodiments, ADC with an average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody. In some instances, even one molecule of toxin/antibody is expected to enhance cytotoxicity over the use of naked antibody.

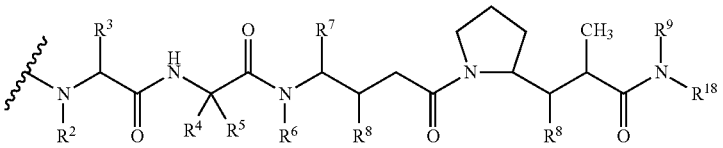
Exemplary linking groups for making antibody-maytansinoid conjugates include, for example, those described herein and those disclosed in U.S. Pat. No. 5,208,020; EP Patent 0 425 235 B1; Chari et al. *Cancer Research* 52:127-131 (1992); US 2005/0276812 A1; and US 2005/016993 A1, the disclosures of which are hereby expressly incorporated by reference.

(2) Auristatins and Dolastatins

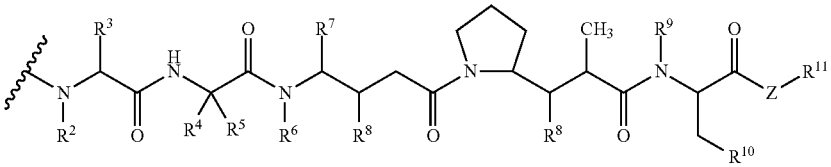
Drug moieties include dolastatins, auristatins, and analogs and derivatives thereof (U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; U.S. Pat. No. 5,767,237; U.S. Pat. No. 6,124,431). Auristatins are derivatives of the marine mollusk compound dolastatin-10. While not intending to be bound by any particular theory, dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) *Antimicrob. Agents and Chemother.* 45(12):3580-3584) and have anticancer (U.S. Pat. No. 5,663,149) and antifungal activity (Pettit et al (1998) *Antimicrob. Agents Chemother.* 42:2961-2965). The dolastatin/auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172; Doronina et al (2003) *Nature Biotechnology* 21(7):778-784; Francisco et al (2003) *Blood* 102(4):1458-1465).

Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties D_E and D_F, disclosed in U.S. Pat. No. 7,498,298 and U.S. Pat. No. 7,659,241, the disclosures of which are expressly incorporated by reference in their entirety:

D_E



D_F



wherein the wavy line of D_E and D_F indicates the covalent attachment site to an antibody or antibody-linker component, and independently at each location:

R^2 is selected from H and C_1-C_8 alkyl;

R^3 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^4 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^5 is selected from H and methyl;

or R^4 and R^5 jointly form a carbocyclic ring and have the formula $-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from H, C_1-C_8 alkyl and C_3-C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

R^6 is selected from H and C_1-C_8 alkyl;

R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle and O—(C_1-C_8 alkyl);

R^9 is selected from H and C_1-C_8 alkyl;

R^{10} is selected from aryl or C_3-C_8 heterocycle;

Z is O, S, NH, or NR^{12}, wherein R^{12} is C_1-C_8 alkyl;

R^{11} is selected from H, C_1-C_{20} alkyl, aryl, C_3-C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2-C_8 alkyl;

R^{14} is H or C_1-C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1$-C_8 alkyl;

each occurrence of R^{16} is independently H, C_1-C_8 alkyl, or $-(CH_2)_n-COOH$;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2$-aryl, $-C(R^8)_2-C(R)_2-(C_3$-C_8 heterocycle), and $-C(R^8)_2-C(R)_2-(C_3$-C_8 carbocycle); and

n is an integer ranging from 0 to 6.

In one embodiment, R^3, R^4 and R^7 are independently isopropyl or sec-butyl and

R^5 is —H or methyl. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is —H, and R^7 is sec-butyl.

In yet another embodiment, R^2 and R^6 are each methyl, and R^9 is —H.

In still another embodiment, each occurrence of R^8 is $-OCH_3$.

In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is —H, R^7 is sec-butyl, each occurrence of R^8 is $-OCH_3$, and R^9 is —H.

In one embodiment, Z is —O— or —NH—.

In one embodiment, R^{10} is aryl.

In an exemplary embodiment, R^{10} is -phenyl.

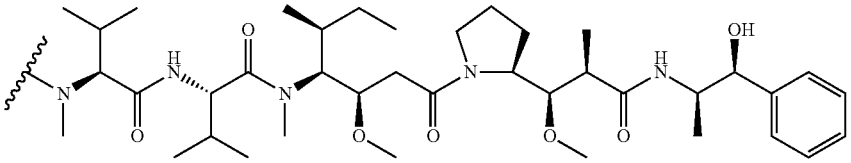
In an exemplary embodiment, when Z is —O—, R^{11} is —H, methyl or t-butyl.

In one embodiment, when Z is —NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is $-C_1$-C_8 alkyl or $-(CH_2)_n-COOH$.

In another embodiment, when Z is —NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

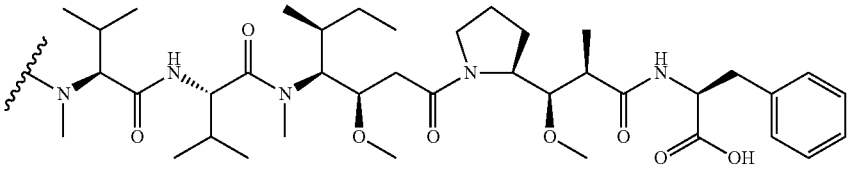
An exemplary auristatin embodiment of formula D_E is MMAE, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:

MMAE



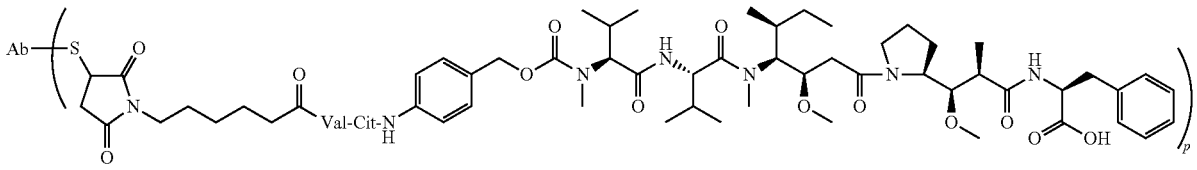
An exemplary auristatin embodiment of formula D_F is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:

MMAF

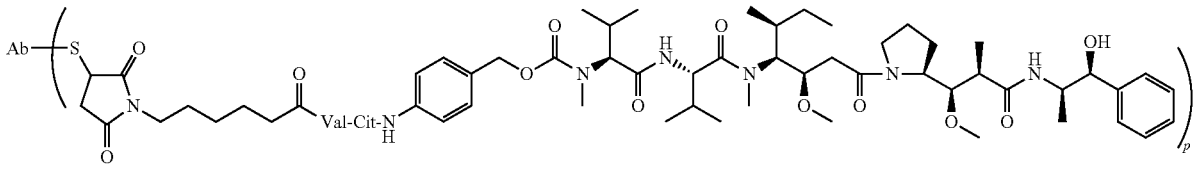


Other exemplary embodiments include monomethylvaline compounds having phenylalanine carboxy modifications at the C-terminus of the pentapeptide auristatin drug moiety (WO 2007/008848) and monomethylvaline compounds having phenylalanine sidechain modifications at the C-terminus of the pentapeptide auristatin drug moiety (WO 2007/008603).

Nonlimiting exemplary embodiments of ADC of Formula I comprising MMAE or MMAF and various linker components have the following structures and abbreviations (wherein "Ab" is an antibody; p is 1 to about 8, "Val-Cit" is a valine-citrulline dipeptide; and "S" is a sulfur atom:



Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE

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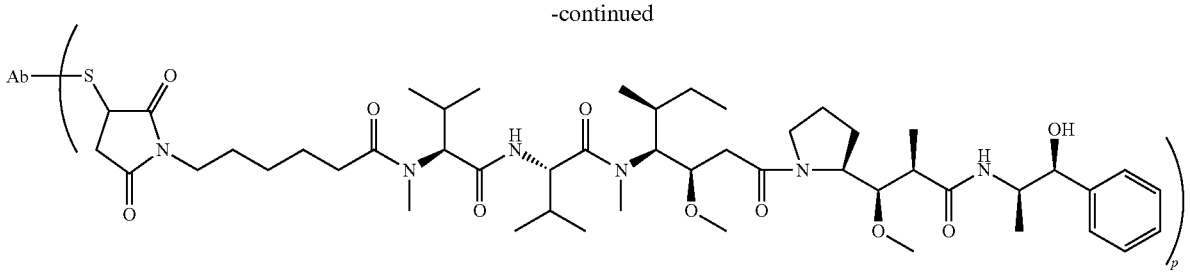
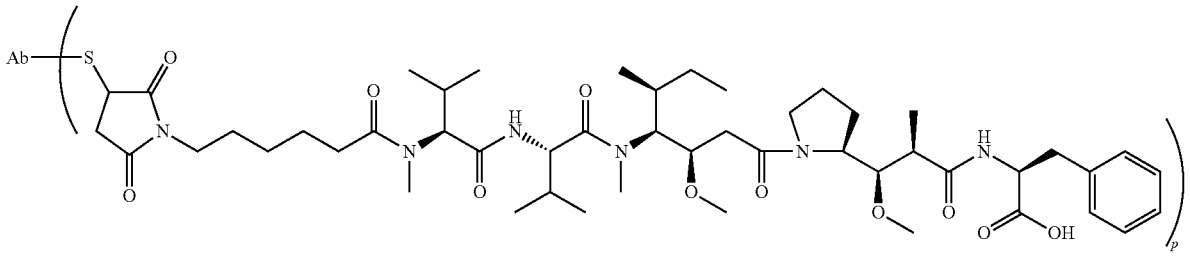


Ab-MC-MMAE



Ab-MC-MMAF

Nonlimiting exemplary embodiments of ADCs of Formula I comprising MMAF and various linker components further include Ab-MC-PAB-MMAF and Ab-PAB-MMAF. Immunoconjugates comprising MMAF attached to an antibody by a linker that is not proteolytically cleavable have been shown to possess activity comparable to immunoconjugates comprising MMAF attached to an antibody by a proteolytically cleavable linker (Doronina et al. (2006) *Bioconjugate Chem.* 17:114-124). In some such embodiments, drug release is believed to be effected by antibody degradation in the cell.

Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to a liquid phase synthesis method (see, e.g., E. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press). Auristatin/dolastatin drug moieties may, in some embodiments, be prepared according to the methods of: U.S. Pat. No. 7,498,298; U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; Pettit et al (1989) *J. Am. Chem. Soc.* 111:5463-5465; Pettit et al (1998) *Anti-Cancer Drug Design* 13:243-277; Pettit, G. R., et al. *Synthesis,* 1996, 719-725; Pettit et al (1996) *J. Chem. Soc. Perkin Trans.* 1 5:859-863; and Doronina (2003) *Nat. Biotechnol.* 21(7):778-784.

In some embodiments, auristatin/dolastatin drug moieties of formulas D_E such as MMAE, and D_F, such as MMAF, and drug-linker intermediates and derivatives thereof, such as MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF, and MC-vc-PAB-MMAE, may be prepared using methods described in U.S. Pat. No. 7,498,298; Doronina et al. (2006) *Bioconjugate Chem.* 17:114-124; and Doronina et al. (2003) *Nat. Biotech.* 21:778-784 and then conjugated to an antibody of interest.

(3) Calicheamicin

In some embodiments, the immunoconjugate comprises an antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics, and analogues thereof, are capable of producing double-stranded DNA breaks at sub-picomolar concentrations (Hinman et al., (1993) *Cancer Research* 53:3336-3342; Lode et al., (1998) *Cancer Research* 58:2925-2928). Calicheamicin has intracellular sites of action but, in certain instances, does not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody-mediated internalization may, in some embodiments, greatly enhances their cytotoxic effects. Nonlimiting exemplary methods of preparing antibody-drug conjugates with a calicheamicin drug moiety are described, for example, in U.S. Pat. No. 5,712,374; U.S. Pat. No. 5,714,586; U.S. Pat. No. 5,739,116; and U.S. Pat. No. 5,767,285.

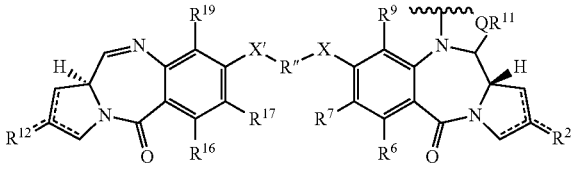
(4) Pyrrolobenzodiazepines

In some embodiments, an ADC comprises a pyrrolobenzodiazepine (PBD). In some embodiments, PDB dimers recognize and bind to specific DNA sequences. The natural product anthramycin, a PBD, was first reported in 1965 (Leimgruber, et al., (1965) *J. Am. Chem. Soc.,* 87:5793-5795; Leimgruber, et al., (1965) *J. Am. Chem. Soc.,* 87:5791-5793). Since then, a number of PBDs, both naturally-occurring and analogues, have been reported (Thurston, et al., (1994) Chem. Rev. 1994, 433-465 including dimers of the tricyclic PBD scaffold (U.S. Pat. No. 6,884,799; U.S. Pat. No. 7,049,311; U.S. Pat. No. 7,067,511; U.S. Pat. No. 7,265,105; U.S. Pat. No. 7,511,032; U.S. Pat. No. 7,528,126; U.S. Pat. No. 7,557,099). Without intending to be bound by any particular theory, it is believed that the dimer structure imparts the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In Antibiotics III. Springer-Verlag, New York, pp. 3-11 (1975); Hurley and Needham-VanDevanter, (1986) *Acc. Chem. Res.,* 19:230-237). Dimeric PBD compounds bearing C2 aryl substituents have been shown to be useful as cytotoxic agents (Hartley et al (2010) *Cancer Res.* 70(17):6849-6858; Antonow (2010) *J. Med. Chem.* 53(7):2927-2941; Howard et al (2009) *Bioorganic and Med. Chem. Letters* 19(22):6463-6466).

PBD dimers have been conjugated to antibodies and the resulting ADC shown to have anti-cancer properties. Nonlimiting exemplary linkage sites on the PBD dimer include the five-membered pyrrolo ring, the tether between the PBD units, and the N10-C11 imine group (WO 2009/016516; US 2009/304710; US 2010/047257; US 2009/036431; US 2011/0256157; WO 2011/130598).

Nonlimiting exemplary PBD dimer components of ADCs are of Formula A:

A



and salts and solvates thereof, wherein:

the wavy line indicates the covalent attachment site to the linker;

the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R^2 is independently selected from H, OH, =O, $=CH_2$, CN, R, OR, $=CH—R^D$, $=C(R^D)_2$, O—SO_2—R, CO_2R and COR, and optionally further selected from halo or dihalo, wherein R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

Q is independently selected from O, S and NH;

R^{11} is either H, or R or, where Q is O, SO_3M, where M is a metal cation;

R and R' are each independently selected from optionally substituted C_{1-8} alkyl, C_{1-12} alkyl, C_{3-8} heterocyclyl, C_{3-20} heterocycle, and C_{5-20} aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

R^{12}, R^{16}, R^{19} and R^{17} are as defined for R^2, R^6, R^9 and R^7 respectively;

R'' is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, N(H), NMe and/or aromatic rings, e.g. benzene or pyridine, which rings are optionally substituted; and

X and X' are independently selected from O, S and N(H).

In some embodiments, R^9 and R^{19} are H.

In some embodiments, R^6 and R^{16} are H.

In some embodiments, R^7 are R^{17} are both OR^{7A}, where R^{7A} is optionally substituted C_{1-4} alkyl. In some embodiments, R^{7A} is Me. In some embodiments, R^{7A} is Ch_2Ph, where Ph is a phenyl group.

In some embodiments, X is O.

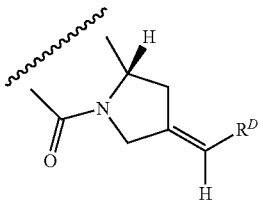
In some embodiments, R^{11} is H.

In some embodiments, there is a double bond between C2 and C3 in each monomer unit.

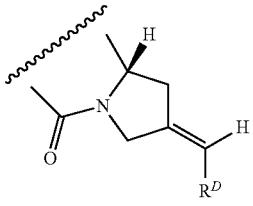
In some embodiments, R^2 and R^{12} are independently selected from H and R. In some embodiments, R^2 and R^{12} are independently R. In some embodiments, R^2 and R^{12} are independently optionally substituted C_{5-20} aryl or C_{5-7} aryl or C_{8-10} aryl. In some embodiments, R^2 and R^{12} are independently optionally substituted phenyl, thienyl, napthyl, pyridyl, quinolinyl, or isoquinolinyl. In some embodiments, R^2 and R^{12} are independently selected from =O, $=CH_2$, $=CH—R^D$, and $=C(RD)_2$. In some embodiments, R^2 and R^{12} each $=CH_2$. In some embodiments, R^2 and R^{12} are each H. In some embodiments, R^2 and R^{12} are each =O. In some embodiments, R^2 and R^{12} are each $=CF_2$. In some embodiments, R^2 and/or R^2 are independently $=C(R^D)_2$. In some embodiments, R^2 and/or R^{12} are independently $=CH—R^D$.

In some embodiments, when R^2 and/or R^{12} is $=CH—R^D$, each group may independently have either configuration shown below:

(I)



(II)

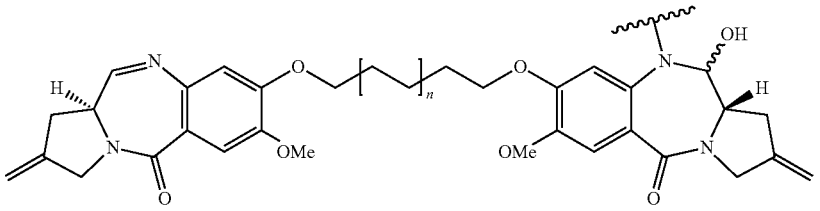


In some embodiments, a $=CH—R^D$ is in configuration (I).

In some embodiments, R is a C_3 alkylene group or a C_5 alkylene group.

In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(I):

A(I)

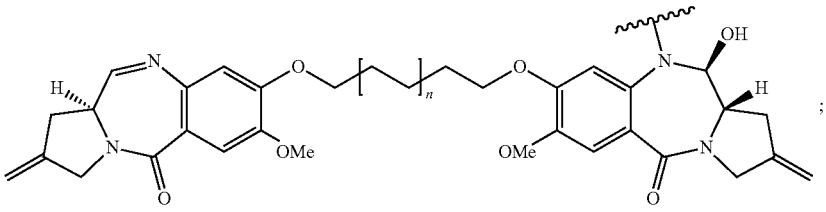


;

wherein n is 0 or 1.

In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(II):

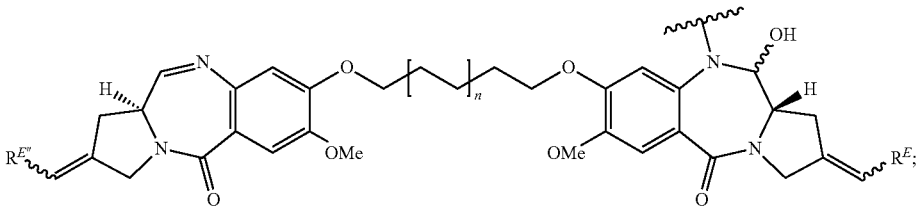
A(II)



wherein n is 0 or 1.

In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(III):

A(III)



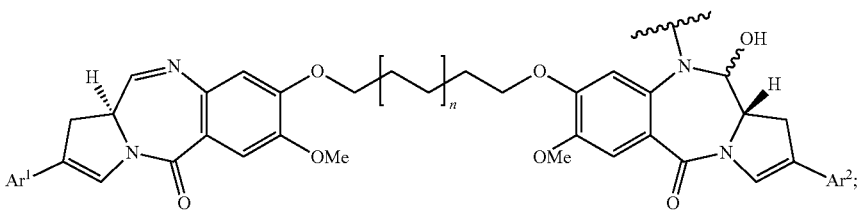
wherein R^E and $R^{E''}$ are each independently selected from H or R^D, wherein R^D is defined as above; and

wherein n is 0 or 1.

In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, R^E and/or $R^{E''}$ is H. In some embodiments, R^E and $R^{E''}$ are H. In some embodiments, R^E and/or $R^{E''}$ is R^D, wherein R^D is optionally substituted C_{1-12} alkyl. In some embodiments, R^E and/or $R^{E''}$ is R^D, wherein R^D is methyl.

In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(IV):

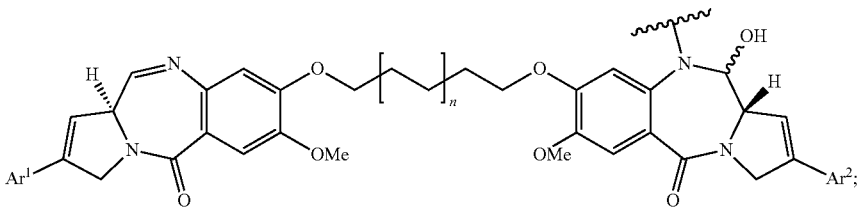
A(IV)



wherein Ar^1 and Ar^2 are each independently optionally substituted C_{5-20} aryl; wherein Ar^1 and Ar^2 may be the same or different; and

wherein n is 0 or 1.

In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(V):



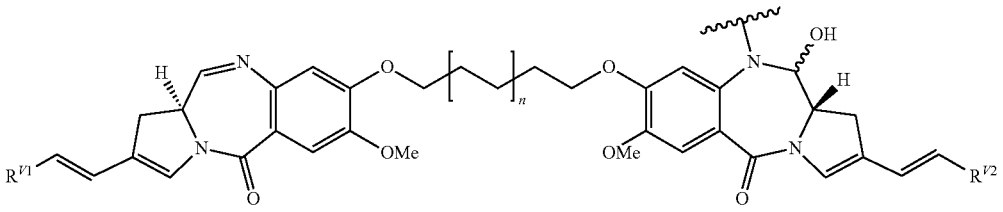
A(V)

wherein Ar^1 and Ar^2 are each independently optionally substituted C_{5-20} aryl; wherein Ar^1 and Ar^2 may be the same or different; and

wherein n is 0 or 1.

In some embodiments, Ar^1 and Ar^2 are each independently selected from optionally substituted phenyl, furanyl, thiophenyl and pyridyl. In some embodiments, Ar^1 and Ar^2 are each independently optionally substituted phenyl. In some embodiments, Ar^1 and Ar^2 are each independently optionally substituted thien-2-yl or thien-3-yl. In some embodiments, Ar^1 and Ar^2 are each independently optionally substituted quinolinyl or isoquinolinyl. The quinolinyl or isoquinolinyl group may be bound to the PBD core through any available ring position. For example, the quinolinyl may be quinolin-2-yl, quinolin-3-yl, quinolin-4-yl, quinolin-5-yl, quinolin-6-yl, quinolin-7-yl and quinolin-8-yl. In some embodiments, the quinolinyl is selected from quinolin-3-yl and quinolin-6-yl. The isoquinolinyl may be isoquinolin-1-yl, isoquinolin-3-yl, isoquinolin-4-yl, isoquinolin-5-yl, isoquinolin-6-yl, isoquinolin-7-yl and isoquinolin-8-yl. In some embodiments, the isoquinolinyl is selected from isoquinolin-3-yl and isoquinolin-6-yl.

Further nonlimiting exemplary PBD dimer components of ADCs are of Formula B:



B

and salts and solvates thereof, wherein:

the wavy line indicates the covalent attachment site to the linker;

the wavy line connected to the OH indicates the S or R configuration;

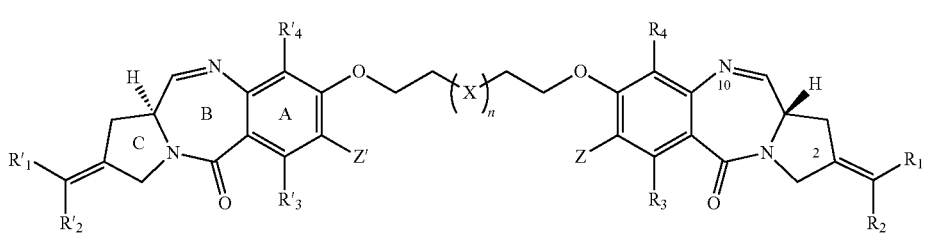
R^{V1} and R^{V2} are independently selected from H, methyl, ethyl and phenyl (which phenyl may be optionally substituted with fluoro, particularly in the 4 position) and C_{5-6} heterocyclyl; wherein R^{V1} and R^{V2} may be the same or different; and

n is 0 or 1.

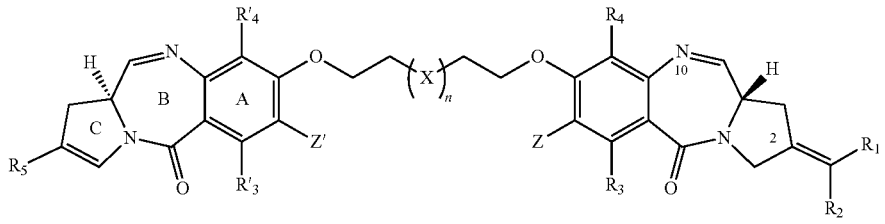
In some embodiments, R^{V1} and R^{V2} are independently selected from H, phenyl, and 4-fluorophenyl.

In some embodiments, a linker may be attached at one of various sites of the PBD dimer drug moiety, including the N10 imine of the B ring, the C-2 endo/exo position of the C ring, or the tether unit linking the A rings (see structures C(I) and C(II) below).

Nonlimiting exemplary PBD dimer components of ADCs include Formulas C(I) and C(II):

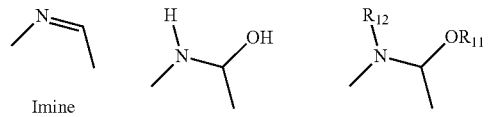


C(I)



C(II)

Formulas C(I) and C(II) are shown in their N10-C11 imine form. Exemplary PBD drug moieties also include the carbinolamine and protected carbinolamine forms as well, as shown in the table below:



Imine	Carbinolamine	Protected Carbinolamine

wherein:

X is CH_2 (n=1 to 5), N, or O;

Z and Z' are independently selected from OR and NR_2, where R is a primary, secondary or tertiary alkyl chain containing 1 to 5 carbon atoms;

R_1, R'_1, R_2 and R'_2 are each independently selected from H, C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_{5-20} aryl (including substituted aryls), C_{5-20} heteroaryl groups, —NH_2, —NHMe, —OH, and —SH, where, in some embodiments, alkyl, alkenyl and alkynyl chains comprise up to 5 carbon atoms;

R_3 and R'_3 are independently selected from H, OR, NHR, and NR_2, where R is a primary, secondary or tertiary alkyl chain containing 1 to 5 carbon atoms;

R_4 and R_{14} are independently selected from H, Me, and OMe;

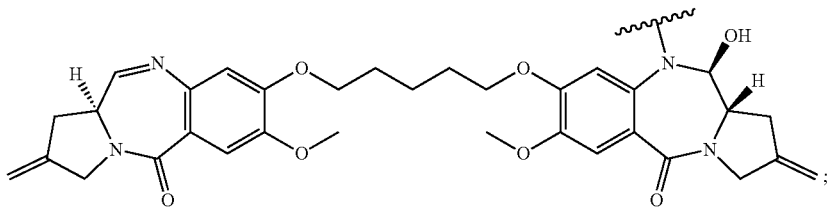
R_5 is selected from C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_{5-20} aryl (including aryls substituted by halo, nitro, cyano, alkoxy, alkyl, heterocyclyl) and C_{5-20} heteroaryl groups, where, in some embodiments, alkyl, alkenyl and alkynyl chains comprise up to 5 carbon atoms;

R_{11} is H, C_1-C_8 alkyl, or a protecting group (such as acetyl, trifluoroacetyl, t-butoxycarbonyl (BOC), benzyloxycarbonyl (CBZ), 9-fluorenylmethylenoxycarbonyl (Fmoc), or a moiety comprising a self-immolating unit such as valine-citrulline-PAB);

R_{12} is H, C_1-C_8 alkyl, or a protecting group;

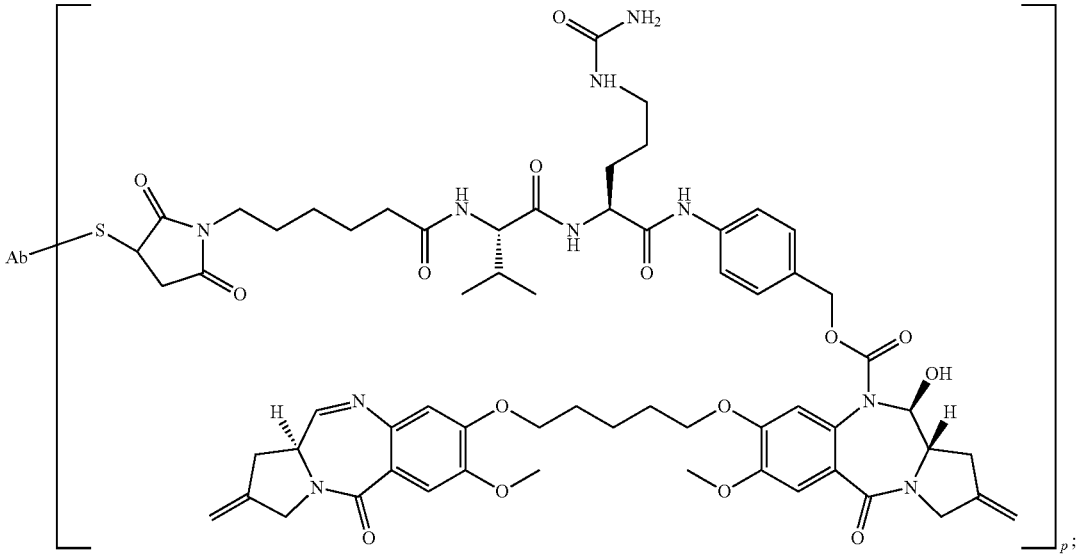
wherein a hydrogen of one of R_1, R'_1, R_2, R'_2, R_5, or R_{12} or a hydrogen of the —$OCH_2CH_2(X)_nCH_2CH_2O$— spacer between the A rings is replaced with a bond connected to the linker of the ADC.

Exemplary PDB dimer portions of ADC include, but are not limited to (the wavy line indicates the site of covalent attachment to the linker):

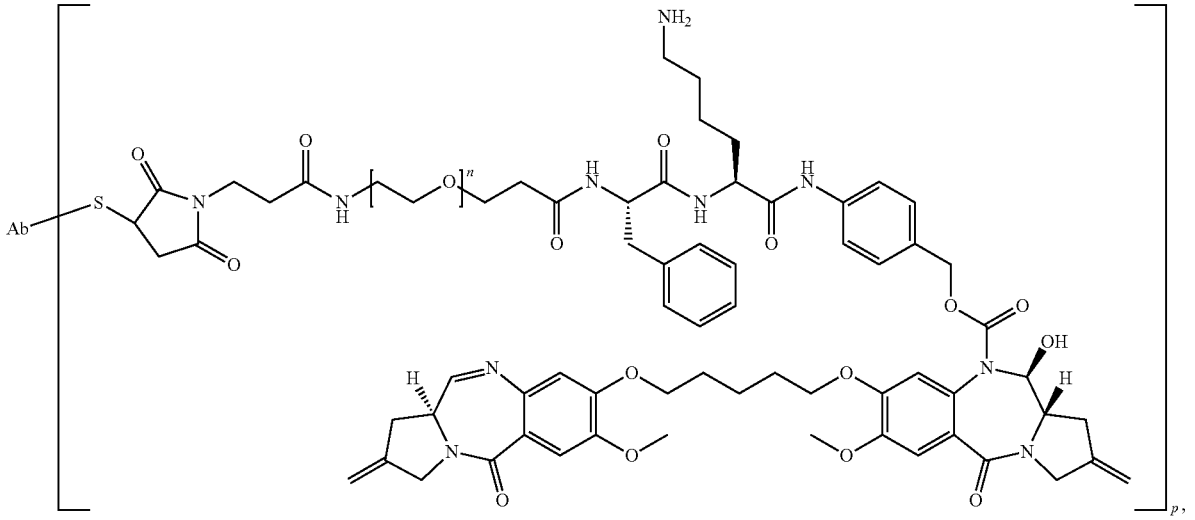


PBD dimer

Nonlimiting exemplary embodiments of ADCs comprising PBD dimers have the following structures:



PBD dimer-val-cit-PAB-Ab



PBD dimer-Phe-Lys-PAB-Ab

n is 0 to 12. In some embodiments, n is 2 to 10. In some embodiments, n is 4 to 8. In some embodiments, n is selected from 4, 5, 6, 7, and 8.

The linkers of PBD dimer-val-cit-PAB-Ab and the PBD dimer-Phe-Lys-PAB-Ab are protease cleavable, while the linker of PBD dimer-maleimide-acetal is acid-labile.

PBD dimers and ADC comprising PBD dimers may be prepared according to methods known in the art. See, e.g., WO 2009/016516; US 2009/304710; US 2010/047257; US 2009/036431; US 2011/0256157; WO 2011/130598.

(5) Anthracyclines

In some embodiments, an ADC comprising anthracycline. Anthracyclines are antibiotic compounds that exhibit cytotoxic activity. While not intending to be bound by any particular theory, studies have indicated that anthracyclines may operate to kill cells by a number of different mechanisms, including: 1) intercalation of the drug molecules into the DNA of the cell thereby inhibiting DNA-dependent nucleic acid synthesis; 2) production by the drug of free radicals which then react with cellular macromolecules to cause damage to the cells, and/or 3) interactions of the drug molecules with the cell membrane (see, e.g., C. Peterson et al., "Transport And Storage Of Anthracycline 1n Experimental Systems And Human Leukemia" in *Anthracycline Antibiotics In Cancer Therapy*; N. R. Bachur, "Free Radical Damage" id. at pp. 97-102). Because of their cytotoxic potential anthracyclines have been used in the treatment of numerous cancers such as leukemia, breast carcinoma, lung carcinoma, ovarian adenocarcinoma and sarcomas (see e.g., P. H-Wiernik, in *Anthracycline: Current Status And New Developments* p 11).

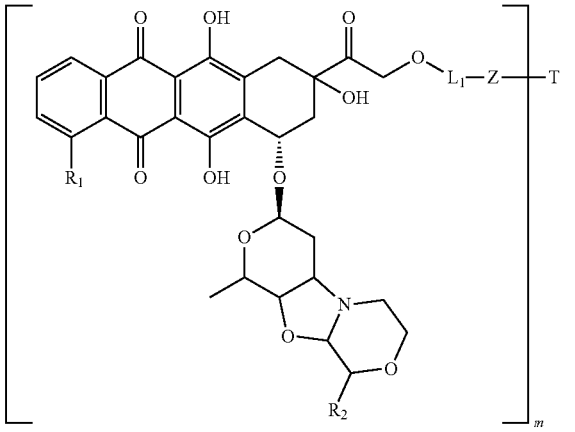
Nonlimiting exemplary anthracyclines include doxorubicin, epirubicin, idarubicin, daunomycin, nemorubicin, and derivatives thereof. Immunoconjugates and prodrugs of daunorubicin and doxorubicin have been prepared and studied (Kratz et al (2006) *Current Med. Chem.* 13:477-523; Jeffrey et al (2006) *Bioorganic & Med. Chem. Letters* 16:358-

362; Torgov et al (2005) *Bioconj. Chem.* 16:717-721; Nagy et al (2000) *Proc. Natl. Acad. Sci. USA* 97:829-834; Dubowchik et al (2002) *Bioorg. & Med. Chem. Letters* 12:1529-1532; King et al (2002) *J. Med. Chem.* 45:4336-4343; EP 0328147; U.S. Pat. No. 6,630,579). The antibody-drug conjugate BR96-doxorubicin reacts specifically with the tumor-associated antigen Lewis-Y and has been evaluated in phase I and II studies (Saleh et al (2000) *J. Clin. Oncology* 18:2282-2292; Ajani et al (2000) *Cancer Jour.* 6:78-81; Tolcher et al (1999) *J. Clin. Oncology* 17:478-484).

PNU-159682 is a potent metabolite (or derivative) of nemorubicin (Quintieri, et al. (2005) *Clinical Cancer Research* 11(4): 1608-1617). Nemorubicin is a semisynthetic analog of doxorubicin with a 2-methoxymorpholino group on the glycoside amino of doxorubicin and has been under clinical evaluation (Grandi et al (1990) *Cancer Treat. Rev.* 17:133; Ripamonti et al (1992) *Brit. J. Cancer* 65:703;), including phase II/III trials for hepatocellular carcinoma (Sun et al (2003) *Proceedings of the American Society for Clinical Oncology* 22, Abs 1448; Quintieri (2003) *Proceedings of the American Association of Cancer Research,* 44:1 st Ed, Abs 4649; Pacciarini et al (2006) *Jour. Clin. Oncology* 24:14116).

A nonlimiting exemplary ADC comprising nemorubicin or nemorubicin derivatives is shown in Formula Ia:

(Ia)



wherein R_1 is hydrogen atom, hydroxy or methoxy group and R_2 is a C_1-C_5 alkoxy group, or a pharmaceutically acceptable salt thereof;

L_1 and Z together are a linker (L) as described herein;

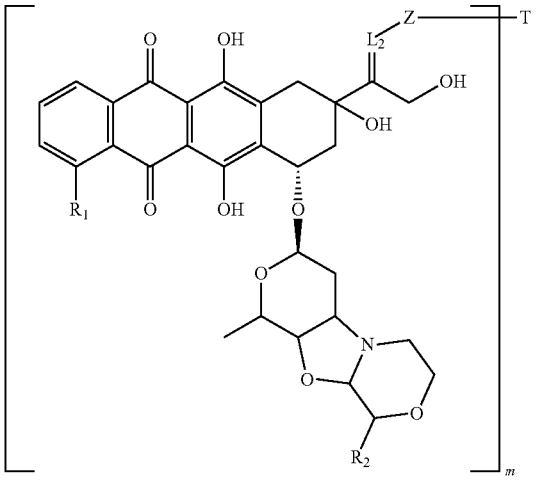
T is an antibody (Ab) as described herein; and

m is 1 to about 20. In some embodiments, m is 1 to 10, 1 to 7, 1 to 5, or 1 to 4.

In some embodiments, R_1 and R_2 are both methoxy (—OMe).

A further nonlimiting exemplary ADC comprising nemorubicin or nemorubicin derivatives is shown in Formula Ib:

(Ib)



wherein R_1 is hydrogen atom, hydroxy or methoxy group and R_2 is a C_1-C_5 alkoxy group, or a pharmaceutically acceptable salt thereof;

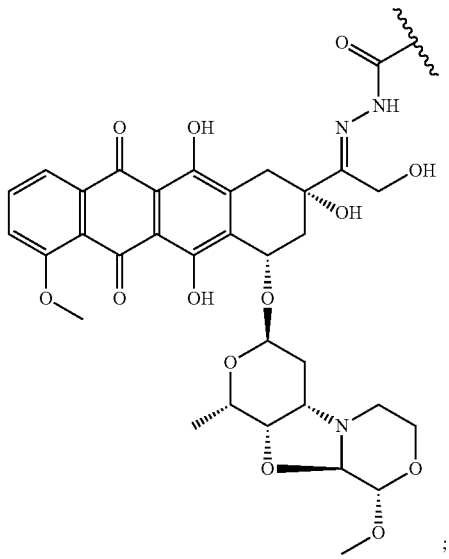
L_2 and Z together are a linker (L) as described herein;

T is an antibody (Ab) as described herein; and

m is 1 to about 20. In some embodiments, m is 1 to 10, 1 to 7, 1 to 5, or 1 to 4.

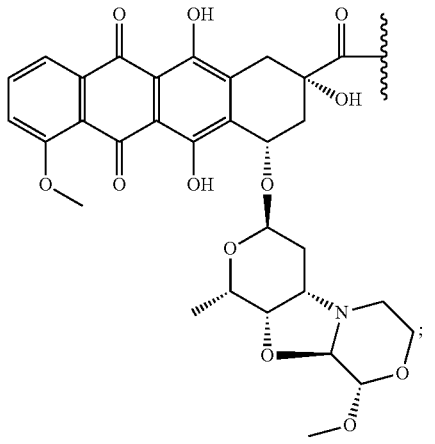
In some embodiments, R_1 and R_2 are both methoxy (—OMe).

In some embodiments, the nemorubicin component of a nemorubicin-containing ADC is PNU-159682. In some such embodiments, the drug portion of the ADC may have one of the following structures:



; or

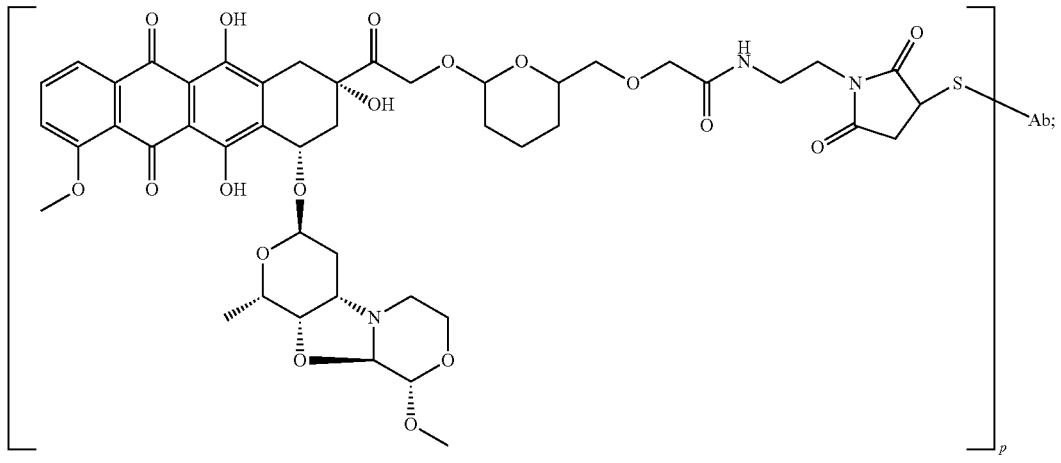
-continued



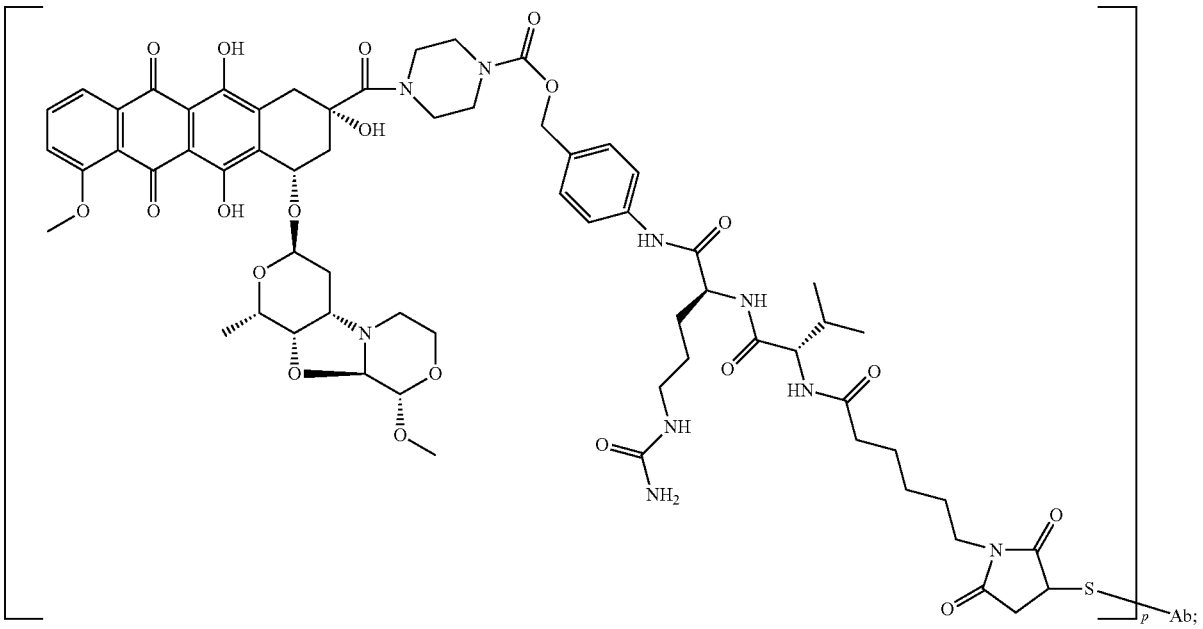
wherein the wavy line indicates the attachment to the linker (L).

Anthracyclines, including PNU-159682, may be conjugated to antibodies through several linkage sites and a variety of linkers (US 2011/0076287; WO2009/099741; US 2010/0034837; WO 2010/009124), including the linkers described herein.

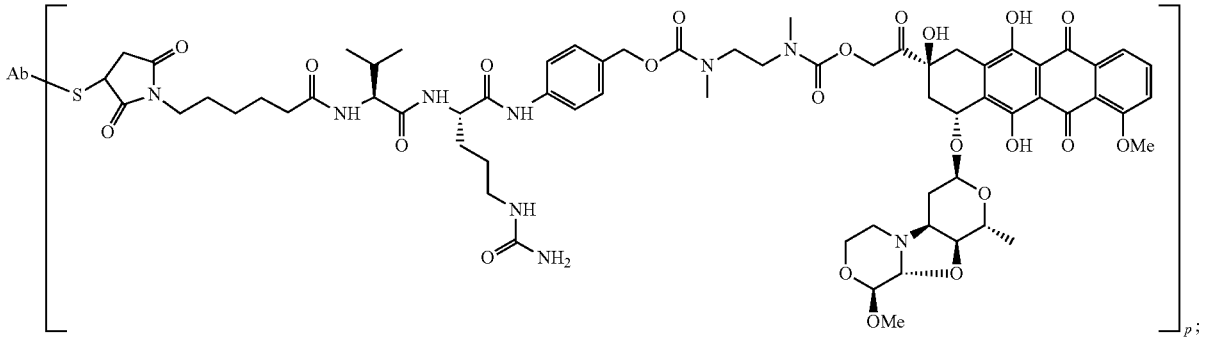
Exemplary ADCs comprising a nemorubicin and linker include, but are not limited to:



PNU-159682 maleimide acetal-Ab

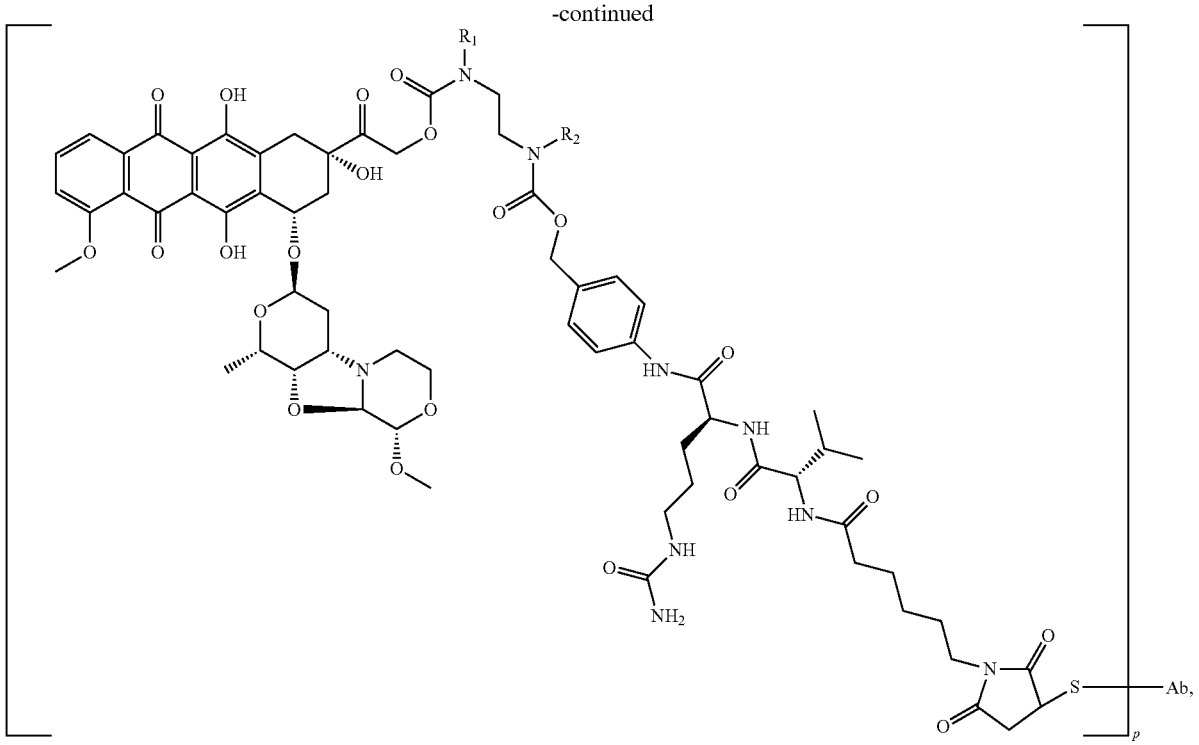


PNU-159682-val-cit-PAB-Ab



PNU-159682-val-cit-PAB-spacer-Ab

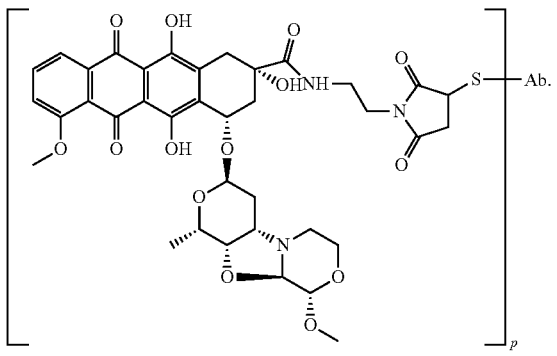
-continued



PNU-159682-val-cit-PAB-spacer(R^1R^2)-Ab

wherein:

R_1 and R_2 are independently selected from H and C_1-C_6 alkyl; and



PNU-159682-maleimide-Ab

The linker of PNU-159682 maleimide acetal-Ab is acid-labile, while the linkers of PNU-159682-val-cit-PAB-Ab, PNU-159682-val-cit-PAB-spacer-Ab, and PNU-159682-val-cit-PAB-spacer(R^1R^2)-Ab are protease cleavable.

(6) Other Drug Moieties

Drug moieties also include geldanamycin (Mandler et al (2000) *J. Nat. Cancer Inst.* 92(19):1573-1581; Mandler et al (2000) *Bioorganic & Med. Chem. Letters* 10:1025-1028; Mandler et al (2002) *Bioconjugate Chem.* 13:786-791); and enzymatically active toxins and fragments thereof, including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, e.g., WO 93/21232.

Drug moieties also include compounds with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease).

In certain embodiments, an immunoconjugate may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. In some embodiments, when an immunoconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc^{99} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, e.g., for PET imaging (WO 2011/056983).

The radio- or other labels may be incorporated in the immunoconjugate in known ways. For example, a peptide may be biosynthesized or chemically synthesized using suitable amino acid precursors comprising, for example, one or more fluorine-19 atoms in place of one or more hydrogens. In some embodiments, labels such as Tc^{99}, I^{123}, R^{186}, Re^{188} and In^{111} can be attached via a cysteine residue in the antibody. In some embodiments, yttrium-90 can be attached via a lysine residue of the antibody. In some embodiments, the IODOGEN method (Fraker et al (1978) *Biochem. Biophys. Res. Commun.* 80: 49-57 can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes certain other methods.

In certain embodiments, an immunoconjugate may comprise an antibody conjugated to a prodrug-activating enzyme. In some such embodiments, a prodrug-activating enzyme converts a prodrug (e.g., a peptidyl chemotherapeutic agent, see WO 81/01145) to an active drug, such as an anti-cancer drug. Such immunoconjugates are useful, in some embodiments, in antibody-dependent enzyme-mediated prodrug therapy ("ADEPT"). Enzymes that may be conjugated to an antibody include, but are not limited to, alkaline phosphatases, which are useful for converting phosphate-containing prodrugs into free drugs; arylsulfatases, which are useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase, which is useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as *serratia* protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), which are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, which are useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase, which are useful for converting glycosylated prodrugs into free drugs; β-lactamase, which is useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase and penicillin G amidase, which are useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. In some embodiments, enzymes may be covalently bound to antibodies by recombinant DNA techniques well known in the art. See, e.g., Neuberger et al., *Nature* 312:604-608 (1984).

c) Drug Loading

Drug loading is represented by p, the average number of drug moieties per antibody in a molecule of Formula I. Drug loading may range from 1 to 20 drug moieties (D) per antibody. ADCs of Formula I include collections of antibodies conjugated with a range of drug moieties, from 1 to 20. The average number of drug moieties per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of ADC in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in certain exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, e.g. p>5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the average drug loading for an ADC ranges from 1 to about 8; from about 2 to about 6; or from about 3 to about 5. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5 (U.S. Pat. No. 7,498,298).

In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the drug-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

The loading (drug/antibody ratio) of an ADC may be controlled in different ways, and for example, by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent, then the resulting product is a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography (see, e.g., McDonagh et al (2006) Prot. Engr. Design & Selection 19(7):299-307; Hamblett et al (2004) Clin. Cancer Res. 10:7063-7070; Hamblett, K. J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, Mar. 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S. C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, Mar. 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In certain embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

d) Certain Methods of Preparing Immunoconjugates

An ADC of Formula I may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent to form Ab-L via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with a nucleophilic group of an antibody. Exemplary methods for preparing an ADC of Formula I via the latter route are described in U.S. Pat. No. 7,498,298, which is expressly incorporated herein by reference.

Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; and (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarbonylethylphosphine (TCEP), such that the anti-

body is fully or partially reduced. Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through modification of lysine residues, e.g., by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may also be introduced into an antibody by introducing one, two, three, four, or more cysteine residues (e.g., by preparing variant antibodies comprising one or more non-native cysteine amino acid residues).

Antibody-drug conjugates of the invention may also be produced by reaction between an electrophilic group on an antibody, such as an aldehyde or ketone carbonyl group, with a nucleophilic group on a linker reagent or drug. Useful nucleophilic groups on a linker reagent include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. In one embodiment, an antibody is modified to introduce electrophilic moieties that are capable of reacting with nucleophilic substituents on the linker reagent or drug. In another embodiment, the sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the antibody that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, antibodies containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, (1992) *Bioconjugate Chem.* 3:138-146; U.S. Pat. No. 5,362,852). Such an aldehyde can be reacted with a drug moiety or linker nucleophile.

Exemplary nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

Nonlimiting exemplary cross-linker reagents that may be used to prepare ADC are described herein in the section titled "Exemplary Linkers." Methods of using such cross-linker reagents to link two moieties, including a proteinaceous moiety and a chemical moiety, are known in the art. In some embodiments, a fusion protein comprising an antibody and a cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. A recombinant DNA molecule may comprise regions encoding the antibody and cytotoxic portions of the conjugate either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

In yet another embodiment, an antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a drug or radionucleotide).

E. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-PMEL17 antibodies provided herein is useful for detecting the presence of PMEL17 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. A "biological sample" comprises, e.g., a cell or tissue (e.g., biopsy material, including cancerous or potentially cancerous skin tissue, including possible or confirmed melanoma; biopsy material from lymphoma, such as cutaneous T-cell lymphoma; and biopsy material from kidney tumors).

In one embodiment, an anti-PMEL17 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of PMEL17 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-PMEL17 antibody as described herein under conditions permissive for binding of the anti-PMEL17 antibody to PMEL17, and detecting whether a complex is formed between the anti-PMEL17 antibody and PMEL17 in the biological sample. Such method may be an in vitro or in vivo method. In one embodiment, an anti-PMEL17 antibody is used to select subjects eligible for therapy with an anti-PMEL17 antibody, e.g. where PMEL17 is a biomarker for selection of patients. In a further embodiment, the biological sample is a cell or tissue (cancerous or potentially cancerous skin tissue, including possible or confirmed melanoma; biopsy material from lymphoma, such as cutaneous T-cell lymphoma; and biopsy material from kidney tumors).

In a further embodiment, an anti-PMEL17 antibody is used in vivo to detect, e.g., by in vivo imaging, a PMEL17-positive cancer in a subject, e.g., for the purposes of diagnosing, prognosing, or staging cancer, determining the appropriate course of therapy, or monitoring response of a cancer to therapy. One method known in the art for in vivo detection is immuno-positron emission tomography (immuno-PET), as described, e.g., in van Dongen et al., *The Oncologist* 12:1379-1389 (2007) and Verel et al., *J. Nucl. Med.* 44:1271-1281 (2003). In such embodiments, a method is provided for detecting a PMEL17-positive cancer in a subject, the method comprising administering a labeled anti-PMEL17 antibody to a subject having or suspected of having a PMEL17-positive cancer, and detecting the labeled anti-PMEL17 antibody in the subject, wherein detection of the labeled anti-PMEL17 antibody indicates a PMEL17-positive cancer in the subject. In certain of such embodiments, the labeled anti-PMEL17 antibody comprises an anti-PMEL17 antibody conjugated to a positron emitter, such as ^{68}Ga, ^{18}F, ^{64}Cu, ^{86}Y, ^{76}Br, ^{89}Zr, and ^{124}I. In a particular embodiment, the positron emitter is ^{89}Zr.

In further embodiments, a method of diagnosis or detection comprises contacting a first anti-PMEL17 antibody immobilized to a substrate with a biological sample to be tested for the presence of PMEL17, exposing the substrate to a second anti-PMEL17 antibody, and detecting whether the second anti-PMEL17 is bound to a complex between the first anti-PMEL17 antibody and PMEL17 in the biological sample. A substrate may be any supportive medium, e.g., glass, metal, ceramic, polymeric beads, slides, chips, and other substrates. In certain embodiments, a biological sample comprises a cell or tissue (cancerous or potentially cancerous skin tissue, including possible or confirmed melanoma; biopsy material from a lymphoma, such as cutaneous T-cell lymphoma; and biopsy material from a kidney tumor). In certain embodiments, the first or second anti-PMEL17 antibody is any of the antibodies described herein.

Exemplary disorders that may be diagnosed or detected according to any of the above embodiments include

PMEL17-positive cancers, such as PMEL17-positive skin cancer (such as melanoma); PMEL17-positive lymphomas (such as cutaneous T-cell lymphoma); and PMEL17-positive kidney tumors. In some embodiments, a PMEL17-positive cancer is a cancer that receives an anti-PMEL17 immunohistochemistry (IHC) score greater than "0," which corresponds to very weak or no staining in >90% of tumor cells, under the conditions described herein in Example K. In some embodiments, a PMEL17-positive cancer expresses PMEL17 at a 1+, 2+ or 3+ level, as defined under the conditions described herein in Example K, using the 31D1 antibody (the 31D1-expressing hybridoma was deposited at the ATCC as 7509 (31D1.6.7) on Apr. 24, 2012). In some embodiments, a PMEL17-positive cancer is a cancer that expresses PMEL17 according to an in situ hybridization (ISH) assay. In some such embodiments, a scoring system similar to that used for IHC is used. In some embodiments, a PMEL17-positive cancer is a cancer that expresses PMEL17 according to a reverse-transcriptase PCR (RT-PCR) assay that detects PMEL17 mRNA. In some embodiments, the RT-PCR is quantitative RT-PCR.

In certain embodiments, labeled anti-PMEL17 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like. In another embodiment, a label is a positron emitter. Positron emitters include but are not limited to ^{68}Ga, ^{18}F, ^{64}Cu, ^{86}Y, ^{76}Br, ^{89}Zr, and ^{124}I. In a particular embodiment, a positron emitter is 89Zr.

F. Pharmaceutical Formulations

Pharmaceutical formulations of an anti-PMEL17 antibody or immunoconjugate as described herein are prepared by mixing such antibody or immunoconjugate having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody or immunoconjugate formulations are described in U.S. Pat. No. 6,267,958. Aqueous antibody or immunoconjugate formulations include those described in U.S. Pat. No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredient as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or immunoconjugate, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

G. Therapeutic Methods and Compositions

Any of the anti-PMEL17 antibodies or immunoconjugates provided herein may be used in methods, e.g., therapeutic methods.

In one aspect, an anti-PMEL17 antibody or immunoconjugate provided herein is used in a method of inhibiting proliferation of a PMEL17-positive cell, the method comprising exposing the cell to the anti-PMEL17 antibody or immunoconjugate under conditions permissive for binding of the anti-PMEL17 antibody or immunoconjugate to PMEL17 on the surface of the cell, thereby inhibiting the proliferation of the cell. In certain embodiments, the method is an in vitro or an in vivo method. In some embodiments, the cell is a melanoma cell. In some embodiments, the cell is a lymphoma cell, such as a cutaneous T cell lymphoma cell. In some embodiments, the cell is a kidney tumor cell.

Inhibition of cell proliferation in vitro may be assayed using the CellTiter-Glo™ Luminescent Cell Viability Assay, which is commercially available from Promega (Madison, Wis.). That assay determines the number of viable cells in culture based on quantitation of ATP present, which is an indication of metabolically active cells. See Crouch et al. (1993) *J. Immunol. Meth.* 160:81-88, U.S. Pat. No. 6,602,677. The assay may be conducted in 96- or 384-well format, making it amenable to automated high-throughput screening

(HTS). See Cree et al. (1995) *AntiCancer Drugs* 6:398-404. The assay procedure involves adding a single reagent (CellTiter-Glo® Reagent) directly to cultured cells. This results in cell lysis and generation of a luminescent signal produced by a luciferase reaction. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable cells present in culture. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is expressed as relative light units (RLU).

In another aspect, an anti-PMEL17 antibody or immunoconjugate for use as a medicament is provided. In further aspects, an anti-PMEL17 antibody or immunoconjugate for use in a method of treatment is provided. In certain embodiments, an anti-PMEL17 antibody or immunoconjugate for use in treating PMEL17-positive cancer is provided. In certain embodiments, the invention provides an anti-PMEL17 antibody or immunoconjugate for use in a method of treating an individual having a PMEL17-positive cancer, the method comprising administering to the individual an effective amount of the anti-PMEL17 antibody or immunoconjugate. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

In a further aspect, the invention provides for the use of an anti-PMEL17 antibody or immunoconjugate in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of PMEL17-positive cancer. In a further embodiment, the medicament is for use in a method of treating PMEL17-positive cancer, the method comprising administering to an individual having PMEL17-positive cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

In a further aspect, the invention provides a method for treating PMEL17-positive cancer. In one embodiment, the method comprises administering to an individual having such PMEL17-positive cancer an effective amount of an anti-PMEL17 antibody or immunoconjugate. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below.

A PMEL17-positive cancer according to any of the above embodiments may be, e.g., PMEL17-positive skin cancer (including melanoma); PMEL17-positive lymphoms (such as cutaneous T-cell lymphoma); and PMEL17-positive kidney tumors. In some embodiments, a PMEL17-positive cancer is a cancer that receives an anti-PMEL17 immunohistochemistry (1HC) or in situ hybridization (ISH) score greater than "0," which corresponds to very weak or no staining in >90% of tumor cells, under the conditions described herein in Example K. In another embodiment, a PMEL17-positive cancer expresses PMEL17 at a 1+, 2+ or 3+ level, as defined under the conditions described herein in Example K, using the 31D1 antibody (the 31D1-expressing hybridoma was deposited at the ATCC as 7509(31D1.6.7) on Apr. 24, 2012). In some embodiments, a PMEL17-positive cancer is a cancer that expresses PMEL17 according to a reverse-transcriptase PCR (RT-PCR) assay that detects PMEL17 mRNA. In some embodiments, the RT-PCR is quantitative RT-PCR.

In some embodiments, methods of treating an individual having a PMEL17-positive cancer are provided, wherein the PMEL17-positive cancer is resistant to a first therapeutic. In some embodiments, the method comprises administering to the individual an effective amount of an immunoconjugate comprising an antibody that binds to PMEL17. In some embodiments, the PMEL17-positive cancer is melanoma. In some embodiments, the first therapeutic comprises a first antibody that binds an antigen other than PMEL17. In some embodiments, the first therapeutic is a first immunoconjugate comprising a first antibody that binds an antigen other than PMEL17 and a first cytotoxic agent. In some embodiments, the first antibody binds an antigen selected from endothelin B receptor (ETBR), tyrosinase-related protein 1 (TYRP1), cytotoxic T lymphocyte antigen 4 (CTLA-4), and glycoprotein NMB (GPNMB). In some embodiments, the first antibody binds ETBR. In some such embodiments, the first immunoconjugate comprises anti-ETBR antibody hu5E9.v1, such as the immunoconjugate hu5E9.v1-MC-val-cit-PAB-MMAE. See U.S. Publication No. US 2011/0206702. The hypervariable regions (HVRs) of hu5E9.v1 are shown herein, e.g., in SEQ ID NOs: 33 to 38. The heavy and light chain variable regions of hu5E9.v1 are shown herein, e.g., in SEQ ID NOs: 40 and 39, respectively. In some embodiments, the first cytotoxic agent and the cytotoxic agent of the immunoconjugate comprising an antibody that binds to PMEL17 are different. In some such embodiments, the first cytotoxic agent is MMAE (such as, for example, when the first immunoconjugate is hu5E9.v1-MC-val-cit-PAB-MMAE) and the cytotoxic agent of the immunoconjugate comprising an antibody that binds to PMEL17 is selected from a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative. In some embodiments, the cytotoxic agent of the immunoconjugate comprising an antibody that binds to PMEL17 is selected from a pyrrolobenzodiazepine and a nemorubicin derivative.

In some embodiments, methods of treating an individual with cancer are provided, wherein the cancer is resistant to a first therapeutic. In some embodiments, the first therapeutic is a first immunoconjugate comprising a first antibody linked to a first cytotoxic agent through a first linker. In some embodiments, a method of treating an individual with a cancer that is resistant to a first therapeutic (such as a first immunoconjugate) comprises administering a second immunoconjugate comprising a second antibody linked to a second cytotoxic agent through a second linker. In some embodiments, the first antibody and the second antibody bind to different antigens and the first cytotoxic agent and the second cytotoxic agents are the same or different. In some embodiments, the first antibody and the second antibody bind to different antigens that are present on at least some of the same cells. In some embodiments, the first antibody and the second antibody bind to different antigens and the first cytotoxic agent and the second cytotoxic agents are different. In some embodiments, the first antibody and the second antibody bind to the same antigens, and the first cytotoxic agent and the second cytotoxic agent are different. In any of the foregoing embodiments, the first linker and the second linker may be the same or different. In some embodiments, the first antibody and the second antibody bind to different antigens, the first and second linkers are different, and the first and second cytotoxic agents are different.

An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-PMEL17 antibodies or immunoconjugate provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-PMEL17 antibodies or immunoconjugates provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-

PMEL17 antibodies or immunoconjugates provided herein and at least one additional therapeutic agent, e.g., as described below.

Antibodies or immunoconjugates of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody or immunoconjugate of the invention may be co-administered with at least one additional therapeutic agent.

In some embodiments, methods of treating cancer comprise administering an immunoconjugate described herein in combination with a second immunoconjugate comprising an antibody that binds an antigen selected from endothelin B receptor (ETBR), tyrosinase-related protein 1 (TYRP1), cytotoxic T lymphocyte antigen 4 (CTLA-4), and glycoprotein NMB (GPNMB). In some such embodiments, the cytotoxic agent of the second immunoconjugate is selected from MMAE, a calicheamicin, a nemorubicin derivative, and a pyrrolobenzodiazepine. The cytotoxic agent of the immunoconjugae described herein and the cytotoxic agent of the second immunoconjugate may be the same or different. In some embodiments, the cytotoxic agents are different. In some embodiments, each cytotoxic agent is selected from MMAE, a nemorubicin derivative, and a pyrrolobenzodiazepine.

In some embodiments, methods of treating cancer comprise administering an immunoconjugate described herein in combination with a second immunoconjugate comprising an antibody that binds ETBR. In some such embodiments, the cancer is a PMEL17-positive cancer and also an ETBR-positive cancer. Determination of whether a cancer is also ETBR-positive may be carried out by any method, including, but not limited to, the methods described herein for determining whether a cancer is PMEL17-positive, and methods described in U.S. Publication No. US 2011/0206702. In some embodiments, the antibody that binds ETBR is hu5E9.v1, which is described, e.g., in U.S. Publication No. US 2011/0206702. In some embodiments, the antibody that binds ETBR comprises an HVR H1 comprising a sequence of SEQ ID NO: 33, an HVR H2 comprising a sequence of SEQ ID NO: 34, an HVR H3 comprising a sequence of SEQ ID NO: 35, an HVR L1 comprising a sequence of SEQ ID NO: 36, an HVR L2 comprising a sequence of SEQ ID NO: 37, and an HVR L3 comprising a sequence of SEQ ID NO: 38. In some embodiments, the antibody that binds ETBR comprises a heavy chain variable region that comprises the sequence of SEQ ID NO: 40 and a light chain variable region that comprises the sequence of SEQ ID NO: 39.

In some embodiments, methods of treating cancer comprise administering an anti-ETBR-MC-val-cit-PAB-MMAE immunoconjugate, such as hu5E9.v1-MC-val-cit-PAB-MMAE (see, e.g., U.S. Publication No. US 2011/0206702), in combination with an immunoconjugate comprising an anti-PMEL17 antibody, such as those described herein. The immunoconjugate comprising an anti-PMEL17 antibody may comprise the same or different cytotoxic agent as the immunoconjugate comprising an anti-ETBR antibody. In some embodiments, the immunoconjugate comprising an anti-PMEL17 antibody comprises a different cytotoxic agent, such as a nemorubicin derivative or a pyrrolobenzodiazepine, e.g., when the anti-ETBR immunoconjugate comprises MMAE.

Administration "in combination" encompasses combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody or immunoconjugate of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent (such as the anti-ETBR immunoconjugate) and/or adjuvant. In some embodiments, administration of the anti-PMEL17 immunoconjugate and administration of an anti-ETBR immunoconjugate occur within about one month, or within about one, two, or three weeks, or within about one, two, three, four, five, or six days of one another. Antibodies or immunoconjugates of the invention can also be used in combination with radiation therapy.

An antibody or immunoconjugate of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies or immunoconjugates of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody or immunoconjugate need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody or immunoconjugate present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody or immunoconjugate of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody or immunoconjugate, the severity and course of the disease, whether the antibody or immunoconjugate is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody or immunoconjugate, and the discretion of the attending physician. The antibody or immunoconjugate is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1 mg/kg-10 mg/kg) of antibody or immunoconjugate can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody or immunoconjugate would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered inter-

mittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried out using both an immunoconjugate of the invention and an anti-PMEL17 antibody.

H. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the disorder and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody or immunoconjugate of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody or immunoconjugate of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution or dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

I. Deposit of Biological Material

The following biological material has been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Va. 20110-2209, USA (ATCC):

Hybridoma Designation	ATCC No.	Deposit Date
7509(31D1.6.7)	PTA-12862	Apr. 24, 2012

The above-referenced deposited hybridoma produces the 31D1 antibody referred to herein.

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures the maintenance of a viable culture of the deposit for 30 years from the date of deposit and for at least five (5) years after the most recent request for furnishing of a sample of the deposit. The deposit will be made available by the ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc., and the ATCC, which assures that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of the pertinent U.S. patent, assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. §122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. §1.14 with particular reference to 8860G 638).

III. Examples

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

A. Human PMEL17 Gene Expression

For the analysis of PMEL17 mRNA expression in multiple human tumor and normal biopsy samples was analyzed using Affymetrix data obtained from Gene Logic, Inc. (Gaithersburg, Md.). The analysis shown is for probe set ID 209848_s_at, performed using the HGU133 Plus v2 GeneChip on 3879 normal human tissue samples, 1605 human cancer tissue samples (1291 primary and 314 metastatic), and 3872 human non-cancer disease tissue samples. Microarray data were normalized using the Affymetrix MAS (Microarray Analysis Suite) version 5.0 software, with sample expression values scaled to a trimmed mean of 500. Analysis of cultured cell lines was performed in similar fashion.

The results are shown in FIGS. **1**A and B. The scale on the y-axis indicates gene expression levels based on hybridization signal intensity. In FIG. **1**A, dots appear both above and below the line extending from the name of each listed tissue. The dots appearing above the line represent gene expression in normal tissue, and the dots appearing below the line represent gene expression in tumor and diseased tissue. As shown in FIG. **1**A, high-level expression of PMEL17 mRNA was largely restricted to neoplasms derived from skin, and all of these were classified as melanoma. A small number of benign kidney tumors that were also positive were classified as epitheloid angiomyolipomas, and a handful of positive lymphomas were identified as cutaneous T-cell lymphomas. FIG. **1**B shows that PMEL17 is overexpressed in many melanoma cell lines, whereas there was little or no expression in cell lines derived from other human cancers. BC, breast cancer; CRC, colorectal cancer; GB, glioblastoma; NSCLC, non-small cell lung cancer; SC, small cell lung cancer; NHL, Non-Hodgkin's lymphoma; MEL, melanoma; MM, multiple myeloma; OVCA, ovarian cancer; PANC, pancreatic cancer; PROS, prostate cancer. Only 5% of the 487 different cell lines analyzed are identified on the figure.

B. Mouse Monoclonal Antibody Generation

Monoclonal antibodies against human PMEL17 were generated using the following procedures. Two separate groups of Balb/C mice (Charles River Laboratories, Hollister, Calif.) were hyperimmunized with either purified human PMEL17 ECD (amino acids 25 to 591 with a C-terminal Fc expressed in CHO cells) in Ribi adjuvant (Group 1) or with plasmid DNA encoding full length PMEL17 (Group 2) via hydrodynamic tail vein (HTV) injection using a modified version of a previously described protocol (see, e.g., Herweijer, H., and Wolff, J. A. (2003) *Gene Ther* 10(6), 453-458; Liu, F., Song, Y., and Liu, D. (1999) *Gene Ther* 6(7), 1258-1266; and Zhang, et al. (1999) *Hum Gene Ther* 10(10), 1735-1737). Following a final boost with protein (Groupl) or PC3 cells

over-expressing PMEL17 (Group 2) 3 days prior to fusion, B cells from mice demonstrating strong specific binding to PC3 cells by FACS from both groups were electro-fused (BTX ECM 2001, Harvard Apparatus) at a 1:1 ratio with X63-Ag8.653 mouse myeloma cells (American Type Culture Collection, Rockwille, Md.), plated at 100,000 cells/well in culture media containing 1× azaserine and hypoxanthine (HA, Sigma-Aldrich, St. Louis, Mo.) and incubated at 37° C., 7% CO2.

After 10-14 days, the supernatants were harvested and screened by ELISA and FACS. For FACS analysis, melanoma cell lines SK-MEL-23 (provided by Paul Robbins, Center for Cancer Research, Tumor Immunology Section), 1300mel (provided by Paul Robbins, Center for Cancer Research, Tumor Immunology Section), SKMEL5 (ATCC HTB-70), and UACC257 (National Cancer Institute), normal melanocytes (Invitrogen, Carlsbad, Calif.), and the prostate cancer cell line PC3 stably expressing human PMEL17 were contacted with antibody. One antibody from the PMEL17-immunized mice, designated 17A9, reacted strongly by fluorescent activated cell sorting (FACS) with live melanoma cells, normal human melanocytes and a PC3 cell line stably expressing PMEL17 cDNA, but not the parental PC3 cell line. Another antibody, 77E6, obtained from the DNA-immunized mice, was also positive by FACS, although 77E6 reacted with live cell lines expressing PMEL17 somewhat inconsistently and more weakly than 17A9.

Positive clones demonstrating strong PMEL17 specific binding by FACS were then expanded and subcloned by limiting dilution. Following two rounds of subcloning and screening, the final clones were cultured in bioreactors (Integra Biosciences, Chur, Switzerland) and supernatants purified by Protein A affinity chromatography as described previously (Hongo, et al. (2000) *Hybridoma* 19(4), 303-315). Three antibody clones, 8G3, 15F2, 17A9, and 31D1 from the PMEL17 ECD-immunized mice, and one antibody clone, 77E6, from the DNA-immunized mice, were selected for further analysis.

C. Mouse Monoclonal Antibody Cloning

Total RNA was extracted from hybridoma cells producing murine 8G3, 15F2, and 17A9 using standard methods. The variable light (VL) and variable heavy (VH) domains were amplified using RT-PCR with degenerate primers to the heavy and light chains. The forward primers were specific for the N-terminal amino acid sequence of the VL and VH regions. Respectively, the LC and HC reverse primers were designed to anneal to a region in the constant light (CL) and constant heavy domain 1 (CH1), which are highly conserved across species. The polynucleotide sequence of the inserts was determined using routine sequencing methods. The 17A9 VH and VL amino acid sequences are shown in SEQ ID NOs: 50 and 2, respectively. The heavy chain hypervariable regions (HVRs) H1, H2, and H3 are shown in SEQ ID NOs: 3, 4, and 5, respectively. The light chain hypervariable regions (HVRs) L1, L2, and L3 are shown in SEQ ID NOs: 6, 7, and 8, respectively. The 8G3 VH and VL amino acid sequences are shown in SEQ ID NOs: 49 and 10, respectively. The heavy chain hypervariable regions (HVRs) H1, H2, and H3 are shown in SEQ ID NOs: 13, 14, and 15, respectively. The light chain hypervariable regions (HVRs) L1, L2, and L3 are shown in SEQ ID NOs: 16, 7, and 8, respectively. The 15F2 VH and VL amino acid sequences are shown in SEQ ID NOs: 51 and 12, respectively. The heavy chain hypervariable regions (HVRs) H1, H2, and H3 are shown in SEQ ID NOs: 20, 21, and 22, respectively. The light chain hypervariable regions (HVRs) L1, L2, and L3 are shown in SEQ ID NOs: 23, 24, and 25, respectively.

An alignment of the light chain and heavy chain variable regions of antibodies 8G3, 17A9, and 15F2 are shown in FIGS. **2**A and **2**B, respectively.

D. Monoclonal Antibody Epitope Mapping

All the constructs for epitope mapping were made by PCR amplification of each fragment and subcloning into either pRKtkneo vector with an N-terminal gD tag, or pRKtkneo vector with an N-terminal gD tag and a C-terminal GPI anchor. Transfections were performed using FuGene 6 (Roche Applied Science), and empty vector was used as control. Full-length human PMEL17 was subcloned into pRKtkneo vector with an N-terminal gD tag.

A signal sequence and a gD epitope tag were fused to the N-terminal sequence of a series of deletion mutants, which were then expressed in mammalian cells and the lysates analyzed by immuoblotting for reactivity with 17A9 or 77E6. The N-terminal deletions tested included Δ100, Δ255, Δ292, Δ315, Δ444, Δ515, and Δ567.

In addition, C-terminal deletions with an N-terminal gD tag and a C-terminal GPI anchor were transiently transfected into 293 cells and analyzed for antibody binding by FACS. The C-terminal deletions tested included $PMEL17_{25\text{-}125}$, $PMEL17_{25\text{-}105}$, $PMEL17_{25\text{-}85}$, $PMEL17_{25\text{-}65}$, and $PMEL17_{25\text{-}45}$. Finally, a PMEL17 fragment comprising the region from amino acid 515 through the transmembrane domain, but lacking the cytoplasmic region ("Δ515-C") and a PMEL17 fragment comprising just the cytoplasmic domain with an N-terminal gD tag and a C-terminal GPI anchor were analyzed by by immuoblotting for reactivity with 77E6.

The results of that experiment are shown in FIG. **3**. FIG. **3**A shows a diagram of the N-terminal PMEL17 deletion mutants, and immunoblots showing reactivity with an anti-gD antibody and 77E6. 77E6 reacted with all of the N-terminal deletions tested. FIG. **3**B shows a diagram of the Δ515-C fragment and the cytoplasmid domain fragment, and immunoblots showing reactivity with an anti-gD antibody and 77E6. 77E6 bound to all deletion mutants that contained the cytoplasmic domain, but not to the Δ515-C mutant, which lacks the cytoplasmic domain. These results confirm that 77E6 binds to an epitope within the cytoplasmic domain of PMEL17.

FIG. **3**C shows immunoblots showing reactivity with an anti-gD antibody and 17A6. 17A6 bound only to full-length PMEL17 and not to the Δ225 or A100 mutants. FIG. **3**D shows a diagram of the C-terminal PMEL17 deletion mutants, and FACS analysis of anti-gD, 17A9, and 31D1 antibody binding to those mutants. 17A9 bound only to $PMEL17_{25\text{-}125}$, and not to any of the other mutants, including $PMEL17_{25\text{-}105}$, suggesting that 17A9 binds to an epitope within the region of amino acids 105 to 125 of PMEL17. 31D1 bound to all of the fragments tested, including $PMEL17_{25\text{-}45}$, suggesting that 31D1 binds to an epitope within the region of amino acids 25 to 45 of PMEL17.

E. Subcellular Localization of PMEL17 Epitopes

PMEL17 is known to undergo rather complex processing during its routing to the melanosome, some of which involves proteolytic cleavages ultimately resulting in deposition of limited RPT domain-containing fragments into fibrillar structures within the mature organelle. This processing involves cleavage at Arg-469 by a proprotein convertase, resulting in an N-terminal Ma and a C-terminal MP3 fragment that remain tethered by a disulfide bond. The Mβ fragment is cleaved further by a metallproteinase at Gln-583 and by γ-secretase in the transmembrane region. Thus, regions of PMEL17 containing the cytoplasmic and N-terminal epitopes are dissociated during this processing.

Because PMEL17 undergoes this complex processing, the subcellular distribution of PMEL17 antibody epitopes in fixed permeabilized 928mel cells (provided by Paul Robbins, Center for Cancer Research, Tumor Immunology Section) cells was determined using 77E6 labeled with Cy3 Fluorophor and 14C10 labeled with Alexa 488. Antibody 14C10 binds to the same deletion fragments as 17A9. A large pool of PMEL17 contianing both cytoplasmic (detected by 77E6) and N-terminal epitopes (detected by 14C10) was present in the perinuclear structure, likely the endoplasmic reticulum. However, in extending toward the cell periphery, a downward gradient of staining intensity by 77E6 is evident, followed by an upward gradient of 14C10 staining, which includes reactivity at the cell membrane. These results suggest that the cytoplasmic epitope recognized by 77E6 is removed during trafficking of PMEL17 to the plasma membrane whereas that the N-terminal epitope progresses to the cell surface.

Consistent with the release of cytoplasmic structure during secretory processing, PMEL17 recovered from conditioned media also failed to react with 77E6. When excised from non-reducing SDS-gels, this secreted PMEL17 generated peptide sequences derived from both Mα and Mβ, but not from the cytoplasmic region, as determined by mass spectrometry. Upon reduction with 2-mercaptoethanol, the secreted PMEL17 exhibited enhanced mobility on SDS-gels and Mα, but not Mβ, sequences were generated from this excised band. These results suggest that the secreted PMEL17 contains Mα and Mβ regions tethered by a disulfide, but lacks cytoplasmic sequence.

The predominance of the N-terminal epitope at the cell surface relative to the cytoplasmic epitope is consistent with stronger FACS signals observed with 17A9 over 77E6. Nevertheless, it appears that some portion of the cytoplasmic region is present at the cell surface to account for the FACS signals observed with 77E6. To address this, live cells were reacted with the 77E6 and 17A9 antibodies that were directly labeled with Alexa Fluors 555 and 488, respectively. To demonstrate that the antibodies did not compete with each other for binding to live cells, 928mel cells were incubated with either of the labeled antibodies alone or both together and FACS analysis was performed, gated for the individual fluorophors. The binding of the individual antibodies was unaffected by the presence of the other antibody. Visualization of live-labeled cells revealed uniform cell surface staining with 17A9, consistent with ample N-terminal epitopes accessible at the plasma membrane. By contrast, 77E6 staining was more isolated and patchy and largely non-overlapping with 17A9 reactivity. Further, although 17A9 stained all cells uniformly and consistently, 77E6 staining was variable from cell to cell and the overall degree of staining varied across preparations. Subsequent experiments demonstrated that suspension of cells greatly enhanced the presence of 77E6 staining at the cell surface, suggesting that culture conditions may account for this variability. Overall, the uniform and intense reactivity of 17A9 on melanoma cells makes this antibody a good candidate for drug conjugation.

F. Species Cross-Reactivity

Antibody 17A9 was tested to determine if it cross-reacts with PMEL17 from species other than human. FIG. **9** shows an alignment between human (SEQ ID NO: 26), cynomolgus monkey (SEQ ID NO: 28), rat (SEQ ID NO: 29) and mouse (SEQ ID NO: 31) PMEL17. All of the sequences include the signal sequence except for the cynomolgus monkey PMEL17. Residues that are identical among all four species are indicated by asterisks (*). In the region where the rat sequence has a deletion, residues that are identical among the remaining three species are indicated by a plus (+). Binding to each species of PMEL17 was determined by FACS analysis using 293S cells stably transfected with an N-terminal gD tagged PMEL17 (human, cynomolgus monkey, rat, or mouse PMEL17); stained with 10 μg/ml 17A9 antibody; and detected with R-Phycoerythrin conjugated goat anti-mouse antibody. Untransfected 293 cells do not normally express PMEL17. 17A9 was found to bind to all four species of PMEL17 tested: human, cynomolgus monkey, rat, and mouse.

G. Internalization of Anti-PMEL17 Antibody

One desirable attribute of an ADC target is the ability to internalize the antibody into a degradative compartment in the cell. 1300mel melanoma cells were seeded in Lab-Tek II cell culture treated 4-well chamber slides (Nalge Nunc International), and incubated with PMEL17 mAb, in the presence of lysosomal protease inhibitors leupeptin and pepstatin A (Sigma-Aldrich) at 50 nM and 5 nM respectively, at 2 μg/ml for either 2 hours or 20 hours in a 37° C. incubator with 5% CO_2. Cells were then washed with PBS, and fixed in 4% Para formaldehyde (Polysciences, Inc.) for 5 min. at room temperature and permeabilized with 0.05% saponin (Sigma-Aldrich) in PBS with 0.5% BSA for 5 minutes at 37° C.; cells were then incubated for one hour with 2 μg/ml rabbit polyclonal anti-LAMP 1 (Sigma-Aldrich), an antibody to a lysosomal marker, followed by one hour incubation of Cy3 labeled anti-mouse IgG (Jackson Immuno Research Laboratories, Inc.) and Alexa-488 labeled anti-rabbit IgG (Invitrogen Life Technology). PMEL17 antibodies were also directly labeled with either Alexa Fluoro 555 or Alexa Fluoro 488 (Invitrogen Life Technology), and stained cells after they were fixed and permeablized as above. Leica SP5 confocal microscope (Leica Microsystems) was used for imaging.

As shown in FIG. **4**, considerable overlap of 17A9 and LAMP1 staining was apparent within the cells. Rapid uptake of 17A9 into additional PMEL17+ melanoma cell lines, including 526mel, SK-MEL-5, and UACC257, was consistently observed. These results predict that a 17A9 ADC should effectively internalize, undergo degradation and release drug to kill melanoma cells.

H. Production of Anti-PMEL17 Antibody Drug Conjugates

For larger scale antibody production, antibodies were produced in CHO cells. Vectors coding for VL and VH were transfected into CHO cells and IgG was purified from cell culture media by protein A affinity chromatography.

Anti-PMEL17 antibody-drug conjugate (ADC) comprising a protease-cleavable val-cit linker and the drug MMAE was produced by conjugating 17A9 or ch17A9 (see, e.g., SEQ ID NOs: 47 and 48) to the drug-linker moiety MC-vc-PAB-MMAE, which is depicted herein. Anti-ETBR ADCs comprising a protease-cleavable val-cit linker and the drug MMAE were produced by conjugating ch5E9 (see, e.g., SEQ ID NOs: 43 and 44) or hu5E9.v1 (see, e.g., SEQ ID NOs: 45 and 46) to the drug-linker moiety MC-vc-PAB-MMAE. For convenience, the drug-linker moiety MC-vc-PAB-MMAE is sometimes referred to in these Examples and in the Figures as "vcMMAE" or "VCE."

Prior to conjugation, the antibody was partially reduced with TCEP using standard methods in accordance with the methodology described in WO 2004/010957 A2. The partially reduced antibody was conjugated to the drug-linker moiety using standard methods in accordance with the methodology described, e.g., in Doronina et al. (2003) *Nat. Biotechnol.* 21:778-784 and US 2005/0238649 A1. Briefly, the partially reduced antibody was combined with the drug-linker moiety to allow conjugation of the drug-linker moiety to reduced cysteine residues of the antibody. The conjugation

reaction was quenched by adding excess N-acetyl-cysteine to react with any free linker-drug moiety, and the ADC was purified. The drug load (average number of drug moieties per antibody) for the ADC was determined to be 2.78. The structure of the ADCs comprising val-cit-MMAE linker-drug (also referred to as MC-val-cit-PAB-MMAE) is shown in FIG. **15**A.

Anti-PMEL17 antibody-drug conjugate (ADC) comprising a protease-cleavable val-cit linker and the drug PNU-159682 was produced by conjugating ch17A9 to the drug-linker moiety MC-vc-PAB-PNU-159682, substantially as described above. Anti-ETBR ADC comprising a protease-cleavable val-cit linker and the drug PNU-159682 was produced by conjugating ch5E9 to the drug-linker moiety MC-vc-PAB-PNU-159682, substantially as described above. The structure of the ADCs comprising MC-val-cit-PAB-PNU-159682 is shown in FIG. **15**C.

Anti-PMEL17 antibody-drug conjugate (ADC) comprising an acid-labile acetal linker and the drug PNU-159682 was produced by conjugating ch17A9 to the drug-linker moiety MC-acetal-PNU-159682, substantially as described above. Anti-ETBR ADC comprising an acid-labile acetal linker and the drug PNU-159682 was produced by conjugating ch5E9 to the drug-linker moiety MC-acetal-PNU-159682, substantially as described above. The structure of the ADCs comprising MC-acetal-PNU-159682 is shown in FIG. **15**B.

Anti-PMEL17 antibody-drug conjugate (ADC) comprising non-cleavable linker and the drug PNU-159682 was produced by conjugating ch17A9 to the drug-linker moiety MC-PNU-159682, substantially as described above. Anti-ETBR ADC comprising non-cleavable linker and the drug PNU-159682 was produced by conjugating ch5E9 to the drug-linker moiety MC-PNU-159682, substantially as described above. The structure of the ADCs comprising PNU-159682 is shown in FIG. **15**E.

Antibody drug conjugates (ADCs) comprising a protease-cleavable val-cit linker and a PBD dimer drug are produced by conjugating an antibody to the drug-linker moiety MC-val-cit-PAB-PBD, substantially as described above. The structure of an antibody-MC-val-cit-PAB-PBD is shown in FIG. **15**D.

I. Inhibition of 1n Vitro Cell Proliferation by an Anti-PMEL17 Antibody Drug Conjugate

Proliferation in the presence of 17A9 ADC was assessed using PMEL17+ melanoma cells and PC3 cells stably expressing PMEL17 plated at 2,000 per well in 50 μL of normal growth medium in 96-well clear-bottom plates (PerkinElmer). Twenty-four hours later, an additional 50 μL of culture medium with serial dilutions of 17A9 ADC were added to triplicate wells. Three or five days later, cell numbers were determined using CellTiter-GloII (Promega Corp.) and with an EnVision 2101 Mutilabel Reader (PerkinElmer).

As shown in FIG. **5**, titration of a panel of PMEL17+ melanoma cells with the 17A9 ADC resulted in effective cell killing with IC_{50}'s ranging from 10 to 100 ng/ml of ADC. The prostate cancer cell line, PC3, stably expressing PMEL17 was effectively killed, but no killing of the vector control PC3 parental cell line was noted out to 10 μg/ml of ADC.

While not intending to be bound by any particular theory, the variation in ADC IC_{50} values for the melanoma cell lines could reflect the variation in the levels of PMEL17 accessible to the ADC, although additional factors likely contribute. For example, the 1300mel cell line expresses significantly more cell surface PMEL17 than the SK-MEL-5 cell line, yet the ADC IC_{50} is slightly higher for 1300mel. In addition, variation in response of the cell lines to the released MMAE drug product could be another contributing factor. The IC_{50} values for free MMAE across eight melanoma cell lines ranged from approximately 0.1 to 0.5 nM. While there was not a strict correlation between the response to free drug and 17A9 ADC, a several-fold difference in free drug sensitivity could, in some instances, account for variation in sensitivity to ADC. Indeed, the SK-MEL-5 was more sensitive to free MMAE than 1300mel, which may compensate for having lower levels of cell surface PMEL17 than 1300mel. Other factors, including the relative rate of antibody internalization might also affect the response to the ADC. Accordingly, the ability of four different melanoma cell lines to accumulate and internalize antibody over time was compared. As expected, cells with higher levels of cell surface PMEL17 accumulated more antibody, but the percentage of that amount internalized appeared comparable, indicating little difference in the relative efficiency of uptake.

J. Toxicity of Anti-PMEL17 ADC in Normal Melanocytes

To assess the potential liability of targeting PMEL17 on normal melanocytes, the level of expression of this target in normal human skin was evaluated by immunohistochemistry. Immunohistochemistry (1HC) was performed on 4 m thick formalin-fixed paraffin embedded tissue sections mounted on glass slides. All IHC steps were carried out on the Ventana Discovery XT (Ventana Medical Systems; Tucson, Ariz.) autostainer. Pretreatment was done with Cell Conditioner 1, standard time. Primary antibody, PMEL17 clone 31D1 was used at a concentration of 10 μg/ml and was incubated on slides for 1 hour at 37° C. Ventana Mouse OmniMap (Ventana Medical Systems; AZ) was used as the detection system. Ventana DAB and Hematoxylin II were used for chromogenic detection and counterstain.

The results of that experiment are shown in FIG. **6**A. Staining with PMEL17 antibody 31D1 revealed intermittent reactivity along the basal layer of the dermis, consistent with the distribution of melanocytes in skin.

Normal human melanocytes and SK-MEL-5 melanoma cells were reacted with chimeric 17A9, or no primary antibody, followed by Alexa 488-labeled anti-human IgG and analyzed by flow cytometry. As shown in FIG. **6**B, cultured normal human melanocytes were also FACS+ with antibody 17A9 and the intensity was comparable to that observed with melanoma cell line SK-MEL-5.

Finally, SK-MEL-5 and SK-MEL-23 melanoma cells, or normal human melanocytes, were incubated with serial dilutions of the 17A9 ADC and five days later cell numbers were determined using CellTiter-GloII. As shown in FIG. **6**C, titration of the normal melanocytes with the 17A9 ADC resulted in cell killing with an IC50 nearly 100-fold higher than that observed with melanoma cell lines. These results are consistent with the mechanism of action of the antimitotic drug MMAE and the reduced proliferative capacity of normal melanocytes relative to melanoma cells.

K. Expression of PMEL17 on Human Melanoma Tissue Specimens

The expression of PMEL17 was evaluated across a panel of human melanoma tissue specimines by immunohistochemistry substantially as described above, using both 17A9 and 31D1. Staining was scored on an arbitrary scale ranging from zero, for absent, to 3+ for most intense:

- 0 (negative): very weak or no staining in >90% of melanoma cells
- 1+ (mild): predominant staining pattern is weak
- 2+ (moderate): predominant staining pattern is moderately strong in the majority (>50%) of melanoma cells
- 3+ (strong): predominant staining pattern is strong in the majority (>50%) of melanoma cells

Exemplary staining for each of the levels of the scale is shown in FIG. **7**A, left panels.

Fifty-eight specimens were obtained from 30 primary and 28 metastatic melanomas. As shown in the table at FIG. **7**B, the full spectrum of staining was observed across the 58 human melanoma specimens with the majority scoring positive with either antibody. 83% (48/58) of the human melanoma specimens were PMEL17+ when detected with 31D1, and 64% (37/58) were PMEL17+ when detected with 17A9. Relative to 17A9, the staining with 31D1 was more highly skewed toward the upper end of the scale. Fixed paraffin-embedded pellets of melanoma cell lines were also sectioned and stained them alongside the tissue specimens to gauge their staining intensity relative to actual melanoma. See FIG. **7**A, right panels.

L. Efficacy of Anti-PMEL17 Antibody Drug Conjugates in SK-MEL-23 Melanoma Cell Line Xenograft

All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Ref-Institute of Laboratory Animal Resources, "Guide for the Care and Use of Laboratory Animals", (NIH Publication #85-23). Washington (DC): National Academy Press; 1996.

The efficacy of the anti-PMEL17 ADCs was investigated using an SK-MEL-23 melanoma cell line xenograft model. Female CRL Nu/Nu mice from Charles River Laboratories were inoculated subcutaneously in the dorsal right flank with five million SK- MEL-23 cells in HBSS with Matrigel. SK-MEL-23 cells exhibit a staining intensity of 2+ to 3+. When tumor volumes reached ~200 mm^3 (day 0), animals were randomized into groups of 10 each and administered a single IV injection of 2 or 6 mg/kg 17A9 ADC. MMAE conjugated to anti-GP 120 antibody was used as a control. Tumor volumes were measured twice per week until study end. Tumor volumes were determined using digital calipers (Fred V. Fowler Company, Inc.) using the formula (L×W×W)/2. Tumor growth inhibition (% TGI) was calculated as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to the vehicle, such that % TGI=100×[1−($AUC_{treatment}$/day)/($AUC_{vehicle}$/day)]. Curve fitting was applied to Log_2 transformed individual tumor volume data using a linear mixed-effects model using the R package nlme, version 3.1-96 in R v2.10.1.

As shown in FIG. **8**, the 6 mg/kg dose of 17A9 ADC was found to retard tumor growth for several weeks, while neither dose of the control ADC had an appreciable effect on tumor growth. These results demonstrate robust and specific efficacy with an ADC targeting PMEL17 on a human melanoma tumor xenograft.

M. Efficacy of Anti-PMEL17 Antibody Drug Conjugates in UACC-257X2.2 Melanoma Cells Resistant to anti-ETBR-vc-MMAE

To determine the efficacy of an anti-PMEL17 ADC in melanoma that had developed resistance to another therapeutic, UACC-257X2.2 melanoma cells that are resistant to an anti-endothelin B receptor (ETBR) immunoconjugate comprising a val-cit linker and MMAE drug (anti-ETBR-vc-MMAE, also referred to as anti-EDNRB-vc-MMAE, anti-ETBR-MC-val-cit-PAB-MMAE, etc.; see, e.g., U.S. Publication No. US 2011/0206702) were developed in vivo and in vitro.

For resistance developed in vivo, NCr nude mice (Taconic, Hudson, N.Y.) were inoculated subcutaneously in the dorsal right flank with 5 million UACC-257X2.2 cells in HBSS with Matrigel. UACC-257X2.2 cells are derived from UACC-257 cells (National Cancer Institute) and optimized for growth in vivo as follows. UACC-257 cells were injected subcutaneously in the right flank of female NCr nude mice to induce tumor growth. One tumor was harvested and grown in vitro (referred to as UACC-257X1.2 cell line). The UACC-257X1.2 line was injected again subcutaneously in the right flank of female NCr nude mice to improve the growth of the cell line in vivo. A tumor from the second injection round was collected and again adapted for in vitro growth to generate UACC-257X2.2. The UACC-257X2.2 cell line and tumors derived from this line express ETBR at levels comparable to the parental cell line UACC-257.

Ten mice inoculated with UACC-257X2.2 cells were dosed with 3 mg/kg hu5E9.v1-MC-vc-PAB-MMAE intravenously on day 0 (the heavy chain and light chain sequences for hu5E9.v1 are shown in SEQ ID NOs: 46 and 45, respectively). To determine when the mice would be dosed again, and at what doses, the following was taken into consideration: whether or not tumors re-grew after the initial treatment (i.e., tumors that grew back to initial tumor volume size at day 0), and the rate of re-growth. Frequency of doses administered varied over time but did not exceed 2 doses/week. Intravenous doses did not exceed 300 μL. The range of doses administered were 3 mg/kg, 6 mg/kg, 8 mg/kg, and 10 mg/kg. Dosing was discontinued once a tumor no longer responded (i.e., it showed resistance to) a series of increasing doses.

As shown in FIG. **10**A, the UACC-257X2.2 tumor #359 developed resistance to anti-ETBR-vc-MMAE after about 120 days, whereas tumor #368 developed resistance more slowly. Tumor #359 was harvested and the cells were dissociated for growth in vitro (referred to herein as in vivo-derived resistant UACC-257X2.2 cells).

For resistance developed in vitro, UACC-257X2.2 cells were adapted to increasing concentrations of anti-ETBR-vc-MMAE in culture dishes over the course of two months. FIG. **10**B shows the resistant UACC-257X2.2 cell line derived in vitro (referred to herein as in vitro-derived resistant UACC-257X2.2 cells), which was relatively unaffected by concentrations of anti-ETBR-vc-MMAE up to at least 10 μg/ml. FIG. **10**B also shows the in vitro-derived resistant UACC-257X2.2 cells incubated with a control ADC, α-gD-vc-MMAE, and the parental UACC-257X2.2 cells incubated with α-ETBR-vc-MMAE.

Expression of ETBR and PMEL17 on the surface of the in vivo- and in vitro-derived resistant UACC-257X2.2 cells and the parental UACC-257X2.2 cells was then determined by FACS. Cells were stained with anti-ETBR antibody (hu5E9.v1 or ch5E9) or with anti-PMEL17 antibody (ch17A9), followed by an anti-human Alexa 488 antibody conjugate. As shown in FIGS. **11**A and B, both the in vivo-derived and in vitro-derived resistant UACC-257X2.2 cells had decreased expression of ETBR on the surface of the cells relative to the parental cell line. Surface expression of PMEL17 was unchanged in the resistant cells as compared to the parental cells. See FIGS. **11**C and D. The control peak in each figure shows staining with secondary antibody only.

In vivo- and in vitro-derived resistant UACC-257X2.2 cells were then assayed for sensitivity to increasing concentrations of anti-ETBR-vc-MMAE, anti-PMEL17-vc-MMAE, and a control ADC, anti-gD-vc-MMAE. See Example H and FIG. **15**A.

In vitro cell proliferation of the cell lines in the presence of the immunoconjugates was assessed. Cells were plated at 1,500 cells per well in 50 L of normal growth medium in 96-well clear-bottom plates. Twenty-four hours later, an additional 50 L of culture medium with serial dilutions of immunoconjugates were added to triplicate wells. Five days later, cell survival was determined using CellTiter-Glo Luminescent Cell Viability Reagent (G7572, Promega Corporation,

Madison, Wis.) using an EnVision 2101 Mutilabel Reader (Perkin-Elmer, Waltham, Mass.).

The results of that experiment are shown in FIG. **12**. The parental UACC-257X2.2 cell line was sensitive to both anti-ETBR-vc-MMAE and anti-PMEL17-vc-MMAE, with EC50s of 45 ng/mL and 33 ng/mL, respectively. See FIG. **12**A. The parental line was also sensitive to linker-drug (without antibody; MC-vc-PAB-MMAE; referred to as "free drug" in FIG. **12**), with an EC50 of 0.28 nM. The in vivo derived resistant UACC-257X2.2 cells were somewhat less sensitive to anti-PMEL17-vc-MMAE and significantly less sensitive to anti-ETBR-vc-MMAE, with EC50s of 119 ng/mL and 235 ng/mL, respectively. See FIG. **12**B. The in vivo derived resistant UACC-257X2.2 cells were also less sensitive to linker-drug (without antibody), with an EC50 of 0.77 nM. The in vitro derived resistant UACC-257X2.2 cells were significantly less sensitive to anti-PMEL17-vc-MMAE, with an EC50 of 764 ng/mL, and insensitive to anti-ETBR-vc-MMAE. See FIG. **12**C. The in vitro derived resistant UACC-257X2.2 cells were also less sensitive to linker-drug (without antibody), with an EC50 of 3.5 nM. These results suggest that resistance may be developed to both the antibody and drug portions of the ADC.

To determine the effect of the antibody portion of the ADC on resistance in UACC-257X2.2 cells, the in vivo- and in vitro-derived resistant UACC-257X2.2 cells were assayed for sensitivity to ADCs with different linkers and different drugs than were used to develop the resistant cells. The ADCs tested in this experiment were anti-ETBR linked to PNU-159682 through a non-cleavable linker (anti-ETBR-PNU) and anti-PMEL17 linked to PNU-159682 through a non-cleavable linker (anti-PMEL17-PNU). See Example H and FIG. **15**E. An unrelated antibody, anti-gD, linked to PNU-159682 through a non-cleavable linker (anti-gD-PNU) was used as a control.

The results of that experiment are shown in FIG. **13**. The parental UACC-257X2.2 cell line was sensitive to both anti-ETBR-PNU and anti-PMEL17-PNU, with EC50s of 4.2 ng/mL and 5.5 ng/mL, respectively. See FIG. **13**A. The parental line was also sensitive to linker-drug (without antibody; MC-PNU-159682; referred to as "free drug" in FIG. **13**), with an EC50 of 0.18 nM in this experiment. The in vivo derived resistant UACC-257X2.2 cells were as sensitive as the parental line to anti-PMEL17-PNU, but somewhat less sensitive to anti-ETBR-PNU, with EC50s of 4.7 ng/mL and 14.3 ng/mL, respectively. See FIG. **13**B. The in vivo derived resistant UACC-257X2.2 cells were also as sensitive to linker-drug (without antibody) as the parental line, with an EC50 of 0.133 nM. Similar to the in vivo derived resistant UACC-257X2.2 cells, the in vitro derived resistant UACC-257X2.2 cells were as sensitive to anti-PMEL17-PNU, but somewhat less sensitive to anti-ETBR-PNU, with EC50s of 4.8 ng/mL and 14.6 ng/mL, respectively. See FIG. **13**C. The in vitro derived resistant UACC-257X2.2 cells were also as sensitive to linker-drug (without antibody) as the parental line, with an EC50 of 0.15 nM. These results demonstrate the extent of resistance in the in vivo- and in vitro-derived resistant UACC-257X2.2 cells that is attributable to the antibody portion of the ADC (for example, because of a decrease in expression of ETBR), as opposed to resistance to the MMAE drug portion of the ADC. Similar results were obtained with anti-ETBR and anti-PMEL17 linked to PNU-159682 through an acid labile linker.

The in vivo- and in vitro-derived resistant UACC-257X2.2 cells were assayed for sensitivity to anti-ETBR linked to PNU-159682 through the vc linker used in the MMAE immunoconjugate (anti-ETBR-vc-PNU) and anti-PMEL17 linked to PNU-159682 through the vc linker (anti-PMEL17-vc-PNU). See Example H and FIG. **15**C. An unrelated antibody, anti-gD, linked to PNU-159682 through the vc linker (anti-gD-vc-PNU) was used as a control.

The results of that experiment are shown in FIG. **14**. The parental UACC-257X2.2 cell line was sensitive to both anti-ETBR-vc-PNU and anti-PMEL17-vc-PNU, with EC50s of 3 ng/mL and 2.5 ng/mL, respectively. See FIG. **14**A. The parental line was also sensitive to linker-drug (without antibody; MC-vc-PAB-PNU-159682), with an EC50 of 2 nM in this experiment. The in vivo derived resistant UACC-257X2.2 cells were slightly less sensitive than the parental line to anti-PMEL17-vc-PNU, and less sensitive to anti-ETBR-vc-PNU, with EC50s of 6.4 ng/mL and 23 ng/mL, respectively. See FIG. **14**B. The in vivo derived resistant UACC-257X2.2 cells were also slightly less sensitive to linker-drug (without antibody) than the parental line, with an EC50 of 4.2 nM. Similarly, the in vitro derived resistant UACC-257X2.2 cells were less sensitive to anti-PMEL17-PNU and anti-ETBR-PNU, with EC50s of 11.6 ng/mL and 41 ng/mL, respectively. See FIG. **14**C. The in vitro derived resistant UACC-257X2.2 cells were also slightly less sensitive to linker-drug (without antibody) than the parental line, with an EC50 of 6.6 nM. These results suggest that the linker component of the ADC may contribute to the resistance in the in vivo- and in vitro-derived resistant UACC-257X2.2 cells. Compare, e.g., the sensitivity of the parental line and in vitro-derived resistant line to anti-PMEL17-vc-PNU (EC50s 2.5 ng/ml and 11.6 ng/ml, respectively), which has a different antibody and drug but the same linker as the ADC used to develop the resistant lines, versus the sensitivity of the parental line and in vitro-derived resistant line to anti-PMEL17-PNU (EC50s 5.5 ng/ml and 4.8 ng/ml, respectively), which has a different antibody, drug, and linker.

The in vivo- and in vitro-derived resistant UACC-257X2.2 cells were assayed for sensitivity to anti-ETBR linked to a PBD dimer through the vc linker used in the MMAE immunoconjugate (anti-ETBR-vc-PBD). The vc-PBD had the structure shown as "PBD dimer-val-cit-PAB-Ab" herein. See also Example H and FIG. **15**D. In that experiment, similar to the results with anti-ETBR-vc-PNU, the resistant cell lines were less sensitive to anti-ETBR-vc-PBD than the parental line, although they were not completely resistant, suggesting that some of the resistance is due to the antibody and linker portions, but some sensitivity can be restored by changing the drug portion of the immunoconjugate.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

TABLE OF SEQUENCES

SEQ ID NO	Description	Sequence
1	17A9 heavy chain variable region	EVQLQQSGPE LVKPGASMKI SCKSSGYSFT RYTMNWVKQS HGKNLEWIGV INPYNGGTVY NQKFKGKATL TVDKSSSTAY MELLSLTSED SAVYYCARTD YDGYAMDYWG QGTSVTVSSA KT
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4	17A9 HVR H2	VINPYNGGTVYNQKFKG
5	17A9 HVR H3	TDYDGYAMDY
6	17A9 HVR L1	RATKSISKYLA
7	17A9 HVR L2; 8G3 HVR L2	SGSTLQS
8	17A9 HVR L3; 8G3 HVR L3	QQHNEYPYT
9	8G3 heavy chain variable region	EVQLQQSGPE LVKPGASMRI SCKASGYSFT GYTMNWVKQS HGKNLEWIGV YNPYNGGTVY NQKFKGKATL TVDKSSSTTY MELLSLTSED SAVYYCARTD SGGYAMDCWG QGTSVTVSSA KT
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11	15F2 heavy chain variable region	QVQLKESGPG LVAPSQSLSI TCTVSGFSLT KYGVHWVRQP PGKGLEWLGV IWAGGNTNYN SALMSRLSIN KDNSKSQVFL KMNSLQTDDT AMYYCATFDV WGAGTTVTVS SAKT
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13	8G3 HVR H1	GYSFTGYTMN
14	8G3 HVR H2	VYNPYNGGTVYNQKFKG
15	8G3 HVR H3	TDSGGYAMDC
16	8G3 HVR L1	RASKTISKYLA
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18	8G3/17A9 HVR H2 consensus	VX$_2$NPYNGGTVYNQKFKG
19	8G3/17A9 HVR L1 consensus	RAX$_3$KX$_4$ISKYLA
20	15F2 HVR H1	GFSLTKYGVH
21	15F2 HVR H2	VIWAGGNTNYNSALMS
22	15F2 HVR H3	TFDV
23	15F2 HVR L1	RSSKSLLHSNGNTFLY
24	15F2 HVR L2	RMSNLVS
25	15F2 HVR L3	MQHLEYPYT
26	Human PMEL17 preproprotein; NP_008859; signal sequence = amino acids 1 to 24	MDLVLKRCLL HLAVIGALLA VGATKVPRNQ DWLGVSRQLR TKAWNRQLYP EWTEAQRLDC WRGGQVSLKV SNDGPTLIGA NASFSIALNF PGSQKVLPDG QVIWVNNTII NGSQVWGGQP VYPQETDDAC IFPDGGPCPS GSWSQKRSFV YVWKTWGQYW QVLGGPVSGL SIGTGRAMLG THTMEVTVYH RRGSRSYVPL AHSSSAFTIT DQVPFSVSVS QLRALDGGNK HFLRNQPLTF ALQLHDPSGY LAEADLSYTW DFGDSSGTLI SRALVVTHTY LEPGPVTAQV VLQAAIPLTS CGSSPVPGTT DGHRPTAEAP NTTAGQVPTT EVVGTTPGQA PTAEPSGTTS VQVPTTEVIS TAPVQMPTAE STGMTPEKVP VSEVMGTTLA EMSTPEATGM TPAEVSIVVL SGTTAAQVTT TEWVETTARE LPIPEPEGPD ASSIMSTESI

TABLE OF SEQUENCES-continued

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27	Human PMEL17 proprotein, without signal sequence; amino acids 25 to 661	KVPRNQ DWLGVSRQLR TKAWNRQLYP EWTEAQRLDC WRGGQVSLKV SNDGPTLIGA NASFSIALNF PGSQKVLPDG QVIWVNNTII NGSQVWGGQP VYPQETDDAC IFPDGGPCPS GSWSQKRSFV YVWKTWGQYW QVLGGPVSGL SIGTGRAMLG THTMEVTVYH RRGSRSYVPL AHSSSAFTIT DQVPFSVSVS QLRALDGGNK HFLRNQPLTF ALQLHDPSGY LAEADLSYTW DFGDSSGTLI SRALVVTHTY LEPGPVTAQV VLQAAIPLTS CGSSPVPGTT DGHRPTAEAP NTTAGQVPTT EVVGTTPGQA PTAEPSGTTS VQVPTTEVIS TAPVQMPTAE STGMTPEKVP VSEVMGTTLA EMSTPEATGM TPAEVSIVVL SGTTAAQVTT TEWVETTARE LPIPEPEGPD ASSIMSTESI TGSLGPLLDG TATLRLVKRQ VPLDCVLYRY GSFSVTLDIV QGIESAEILQ AVPSGEGDAF ELTVSCQGGL PKEACMEISS PGCQPPAQRL CQPVLPSPAC QLVLHQILKG GSGTYCLNVS LADTNSLAVV STQLIMPGQE AGLGQVPLIV GILLVLMAVV LASLIYRRRL MKQDFSVPQL PHSSSHWLRL PRIFCSCPIG ENSPLLSGQQ V
28	Cynomolgus monkey PMEL17, without signal sequence	KGPRNQDWLG VSRQLRTKAW NRQLYPEWTE AQRLDCWRGG QVSLKVSNDG PTLIGANASF SIALNFPGSQ KVLPDGQVIW VNNTIINGSQ VWGGQPVYPQ ETDDACIFPD GGPCPSGPWS QKRSFVYVWK TWGQYWQVLG GPVSGLSIGT GRAMLGTHTM EVTVYHRRGS RSYVPLAHSS SAFTITDQVP FSVSVSQLRA LDGGNKHFLR NQPLTFALQL HDPSGYLAEA DLSYTWDFGD SSGTLISRAL VVTHTYLEPG PVTAQVVLQA AIPLTSCGSS PVPGTTDGHR PTAEAPDTTA GRGPTTEVVG TTPGQVPTTQ PSGTTSVQVP TTEVISTTPV QMPTAESTGT TPEKVPVSEV MGTTLAEMST PEAIGMTPAE VSIVVPSGTT AAQVTTTEWV ETTAGELPTP EPEGPDTSSI MSTESITGSL GPLLDGTATL RLEKRQVPLD CVLYRYGSFS VTLDIVQGIE SAEILQVVPS SEGDAFELTV SCQGGLPTEA CMEISSPGCQ PPAQQLCQPV PPSPACQLVL YQILKGGLGT YCLNVSLADA NSLAVVSTQL IVPGQEAGLG QAPLFVGILL VLMAVVLASL IYRRRLMKQA FSIPQLPHGS SHWLRLPRIF RSCPIGENSP LLSGQEV
29	Rat PMEL17 precursor; NP_068682; signal sequence = amino acids 1 to 25	MGVQRRCFLP VLVLGALLAL GSIEGSRNQN WHGVSRQLVT KVWNKQLYPE WTEVQGSNCW RGGQVSLKVR NDGPTLVGAN TSFSIALHFP GSQKVLPDGQ VIWVNNTIIN GSQVWGGQPV YPREPDDACI FPDGGPCPSG PKPPRRSFVY VWKTWGQYWQ VLGGPESKLS IPTGHARLGT HTMEVTVYHR RGSQSYVPLA HSSSTFTITG SVSRLLDDTD TIMLVKRQVP LDCVLYRYGS FSLTLDIVQG IESAEILQAV PSSEGDAFEL TVSCRGGLPK EACMDISSPG CQPPAQRLCQ PVPPSPDCQL VLHQILKGGL GTYCLNVSLA DANSLAVAST QLVVPGQEGS LGQAPLLVGV LLVLVAVVLA SLIYRHRLKK QDSVSQTPHG STHWLRLPPV FCARRLGESS PLLSGQQV
30	Rat PMEL17, without signal sequence, amino acids 26 to 418	SRNQN WHGVSRQLVT KVWNKQLYPE WTEVQGSNCW RGGQVSLKVR NDGPTLVGAN TSFSIALHFP GSQKVLPDGQ VIWVNNTIIN GSQVWGGQPV YPREPDDACI FPDGGPCPSG PKPPRRSFVY VWKTWGQYWQ VLGGPESKLS IPTGHARLGT HTMEVTVYHR RGSQSYVPLA HSSSTFTITG SVSRLLDDTD TIMLVKRQVP LDCVLYRYGS FSLTLDIVQG IESAEILQAV PSSEGDAFEL TVSCRGGLPK EACMDISSPG CQPPAQRLCQ PVPPSPDCQL VLHQILKGGL GTYCLNVSLA DANSLAVAST QLVVPGQEGS LGQAPLLVGV LLVLVAVVLA SLIYRHRLKK QDSVSQTPHG STHWLRLPPV FCARRLGESS PLLSGQQV
31	Mouse PMEL17 precursor; NP_068682; signal sequence = amino acids 1 to 25	MGVQRRSFLP VLVLSALLAV GALEGSRNQD WLGVPRQLVT KTWNRQLYPE WTEVQGSNCW RGGQVSLRVI NDGPTLVGAN ASFSIALHFP GSQKVLPDGQ VIWANNTIIN GSQVWGGQPV YPQEPDDACV FPDGGPCPSG PKPPKRSFVY VWKTWGKYWQ VLGGPVSRLS IATGHAKLGT HTMEVTVYHR RGSQSYVPLA HASSTFTITD QVPFSVSVSQ LQALDGETKH FLRNHPLIFA LQLHDPSGYL AEADLSYTWD FGDGTGTLIS RALDVTHTYL ESGSVTAQVV LQAAIPLVSC GSSPVPGTTD GYMPTAEAPG TTSRQGTTTK VVGTTPGQMP TTQPSGTTVV QMPTTEVTAT TSEQMLTSAV IDTTLAEVST TEGTGTTPTR PSGTTVAQAT TTEGPDASPL LPTQSSTGSI SPLLDDTDTI MLVKRQVPLD CVLYRYGSFS LALDIVQGIE SAEILQAVPF SEGDAFELTV SCQGGLPKEA CMDISSPGCQ PPAQRLCQSV PPSPDCQLVL HQVLKGGSGT YCLNVSLADA NSLAVASTQL VVPGQDGGLG QAPLLVGILL VLVAVVLASL IHRHRLKKQG SVSQMPHGST HWLRLPPVFR ARGLGENSPL LSGQQV
32	Mouse PMEL17, without signal sequence, amino acids 26 to 626	SRNQD WLGVPRQLVT KTWNRQLYPE WTEVQGSNCW RGGQVSLRVI NDGPTLVGAN ASFSIALHFP GSQKVLPDGQ VIWANNTIIN GSQVWGGQPV YPQEPDDACV FPDGGPCPSG PKPPKRSFVY VWKTWGKYWQ VLGGPVSRLS IATGHAKLGT HTMEVTVYHR RGSQSYVPLA HASSTFTITD QVPFSVSVSQ LQALDGETKH FLRNHPLIFA LQLHDPSGYL AEADLSYTWD FGDGTGTLIS RALDVTHTYL ESGSVTAQVV LQAAIPLVSC GSSPVPGTTD GYMPTAEAPG TTSRQGTTTK VVGTTPGQMP TTQPSGTTVV QMPTTEVTAT TSEQMLTSAV IDTTLAEVST TEGTGTTPTR PSGTTVAQAT TTEGPDASPL LPTQSSTGSI SPLLDDTDTI MLVKRQVPLD CVLYRYGSFS LALDIVQGIE SAEILQAVPF

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35	5E9 HVR H3	WGYAYDIDN
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38	5E9 HVR L3	WQGTHFPYT
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49	8G3 heavy chain variable region	EVQLQQSGPE LVKPGASMRI SCKASGYSFT GYTMNWVKQS HGKNLEWIGV YNPYNGGTVY NQKFKGKATL TVDKSSSTTY MELLSLTSED SAVYYCARTD SGGYAMDCWG QGTSVTVSS
50	17A9 heavy chain variable region	EVQLQQSGPE LVKPGASMKI SCKSSGYSFT RYTMNWVKQS HGKNLEWIGV INPYNGGTVY NQKFKGKATL TVDKSSSTAY MELLSLTSED SAVYYCARTD YDGYAMDYWG QGTSVTVSS
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35 40 45

Gly Val Ile Asn Pro Tyr Asn Gly Gly Thr Val Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
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Ala Arg Thr Asp Tyr Asp Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly
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Thr Ser Val Thr Val Ser Ser Ala Lys Thr
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Leu Ala Trp Tyr Gln Glu Gln Pro Gly Lys Thr Asn Asn Leu Leu Ile
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Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly
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<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 5

Thr Asp Tyr Asp Gly Tyr Ala Met Asp Tyr
1               5                   10


<210> SEQ ID NO 6
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 6

Arg Ala Thr Lys Ser Ile Ser Lys Tyr Leu Ala
1               5                   10


<210> SEQ ID NO 7
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 7

Ser Gly Ser Thr Leu Gln Ser
1               5


<210> SEQ ID NO 8
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 8

Gln Gln His Asn Glu Tyr Pro Tyr Thr
1               5
```

-continued

<210> SEQ ID NO 9
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 9

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Met Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
20 25 30

Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp Ile
35 40 45

Gly Val Tyr Asn Pro Tyr Asn Gly Gly Thr Val Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Thr Tyr
65 70 75 80

Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Thr Asp Ser Gly Gly Tyr Ala Met Asp Cys Trp Gly Gln Gly
100 105 110

Thr Ser Val Thr Val Ser Ser Ala Lys Thr
115 120

<210> SEQ ID NO 10
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 10

Asp Val Gln Ile Thr Gln Ser Pro Ser Tyr Leu Asp Ala Ser Pro Gly
1 5 10 15

Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Thr Ile Ser Lys Tyr
20 25 30

Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile
35 40 45

Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Tyr Pro Tyr
85 90 95

Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105

<210> SEQ ID NO 11
<211> LENGTH: 114
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 11

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Lys Tyr
20 25 30

Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ala Gly Gly Asn Thr Asn Tyr Asn Ser Ala Leu Met
50 55 60

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Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65                  70                  75                  80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
                85                  90                  95

Thr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Ala
            100                 105                 110

Lys Thr


<210> SEQ ID NO 12
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 12

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1               5                   10                  15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
            20                  25                  30

Asn Gly Asn Thr Phe Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Val Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Phe Tyr Tyr Cys Met Gln His
                85                  90                  95

Leu Glu Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys
            100                 105                 110


<210> SEQ ID NO 13
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

Gly Tyr Ser Phe Thr Gly Tyr Thr Met Asn
1               5                   10


<210> SEQ ID NO 14
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 14

Val Tyr Asn Pro Tyr Asn Gly Gly Thr Val Tyr Asn Gln Lys Phe Lys
1               5                   10                  15

Gly


<210> SEQ ID NO 15
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 15

Thr Asp Ser Gly Gly Tyr Ala Met Asp Cys
1               5                   10


<210> SEQ ID NO 16
<211> LENGTH: 11
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-continued

<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 16

Arg Ala Ser Lys Thr Ile Ser Lys Tyr Leu Ala
1 5 10

<210> SEQ ID NO 17
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa is selected from Arg, Lys, Gly, and Ala

<400> SEQUENCE: 17

Gly Tyr Ser Phe Thr Xaa Tyr Thr Met Asn
1 5 10

<210> SEQ ID NO 18
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa is selected from Tyr, Trp, Phe, Thr, Ser,
Ile, Leu, Val, Met, Ala, and Norleucine

<400> SEQUENCE: 18

Val Xaa Asn Pro Tyr Asn Gly Gly Thr Val Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

<210> SEQ ID NO 19
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is selected from Ser, Thr, and Val
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is selected from Ser, Thr, and Val

<400> SEQUENCE: 19

Arg Ala Xaa Lys Xaa Ile Ser Lys Tyr Leu Ala
1 5 10

<210> SEQ ID NO 20
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

Gly Phe Ser Leu Thr Lys Tyr Gly Val His
1 5 10

<210> SEQ ID NO 21
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 21

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Val Ile Trp Ala Gly Gly Asn Thr Asn Tyr Asn Ser Ala Leu Met Ser
1               5                   10                  15


<210> SEQ ID NO 22
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 22

Thr Phe Asp Val
1


<210> SEQ ID NO 23
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 23

Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Phe Leu Tyr
1               5                   10                  15


<210> SEQ ID NO 24
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 24

Arg Met Ser Asn Leu Val Ser
1               5


<210> SEQ ID NO 25
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 25

Met Gln His Leu Glu Tyr Pro Tyr Thr
1               5


<210> SEQ ID NO 26
<211> LENGTH: 661
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Met Asp Leu Val Leu Lys Arg Cys Leu Leu His Leu Ala Val Ile Gly
1               5                   10                  15

Ala Leu Leu Ala Val Gly Ala Thr Lys Val Pro Arg Asn Gln Asp Trp
            20                  25                  30

Leu Gly Val Ser Arg Gln Leu Arg Thr Lys Ala Trp Asn Arg Gln Leu
        35                  40                  45

Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
    50                  55                  60

Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
65                  70                  75                  80

Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
                85                  90                  95

Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
            100                 105                 110

Ser Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Thr Asp Asp
        115                 120                 125
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Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser
    130                 135                 140

Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
145                 150                 155                 160

Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
                165                 170                 175

Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
            180                 185                 190

Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr
        195                 200                 205

Ile Thr Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
    210                 215                 220

Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
225                 230                 235                 240

Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
                245                 250                 255

Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
            260                 265                 270

Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Ala
        275                 280                 285

Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
    290                 295                 300

Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
305                 310                 315                 320

Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
                325                 330                 335

Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
            340                 345                 350

Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr
        355                 360                 365

Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val
    370                 375                 380

Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met
385                 390                 395                 400

Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
                405                 410                 415

Gln Val Thr Thr Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu Pro
            420                 425                 430

Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu
        435                 440                 445

Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu
    450                 455                 460

Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr
465                 470                 475                 480

Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala
                485                 490                 495

Glu Ile Leu Gln Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu Leu
            500                 505                 510

Thr Val Ser Cys Gln Gly Gly Leu Pro Lys Glu Ala Cys Met Glu Ile
        515                 520                 525

Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val
    530                 535                 540

Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly
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545                 550                 555                 560

Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
                565                 570                 575

Leu Ala Val Val Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala Gly
            580                 585                 590

Leu Gly Gln Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu Met Ala
        595                 600                 605

Val Val Leu Ala Ser Leu Ile Tyr Arg Arg Arg Leu Met Lys Gln Asp
    610                 615                 620

Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu
625                 630                 635                 640

Pro Arg Ile Phe Cys Ser Cys Pro Ile Gly Glu Asn Ser Pro Leu Leu
                645                 650                 655

Ser Gly Gln Gln Val
            660


<210> SEQ ID NO 27
<211> LENGTH: 637
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

Lys Val Pro Arg Asn Gln Asp Trp Leu Gly Val Ser Arg Gln Leu Arg
1               5                   10                  15

Thr Lys Ala Trp Asn Arg Gln Leu Tyr Pro Glu Trp Thr Glu Ala Gln
            20                  25                  30

Arg Leu Asp Cys Trp Arg Gly Gly Gln Val Ser Leu Lys Val Ser Asn
        35                  40                  45

Asp Gly Pro Thr Leu Ile Gly Ala Asn Ala Ser Phe Ser Ile Ala Leu
    50                  55                  60

Asn Phe Pro Gly Ser Gln Lys Val Leu Pro Asp Gly Gln Val Ile Trp
65                  70                  75                  80

Val Asn Asn Thr Ile Ile Asn Gly Ser Gln Val Trp Gly Gly Gln Pro
                85                  90                  95

Val Tyr Pro Gln Glu Thr Asp Asp Ala Cys Ile Phe Pro Asp Gly Gly
            100                 105                 110

Pro Cys Pro Ser Gly Ser Trp Ser Gln Lys Arg Ser Phe Val Tyr Val
        115                 120                 125

Trp Lys Thr Trp Gly Gln Tyr Trp Gln Val Leu Gly Gly Pro Val Ser
    130                 135                 140

Gly Leu Ser Ile Gly Thr Gly Arg Ala Met Leu Gly Thr His Thr Met
145                 150                 155                 160

Glu Val Thr Val Tyr His Arg Arg Gly Ser Arg Ser Tyr Val Pro Leu
                165                 170                 175

Ala His Ser Ser Ser Ala Phe Thr Ile Thr Asp Gln Val Pro Phe Ser
            180                 185                 190

Val Ser Val Ser Gln Leu Arg Ala Leu Asp Gly Gly Asn Lys His Phe
        195                 200                 205

Leu Arg Asn Gln Pro Leu Thr Phe Ala Leu Gln Leu His Asp Pro Ser
    210                 215                 220

Gly Tyr Leu Ala Glu Ala Asp Leu Ser Tyr Thr Trp Asp Phe Gly Asp
225                 230                 235                 240

Ser Ser Gly Thr Leu Ile Ser Arg Ala Leu Val Val Thr His Thr Tyr
                245                 250                 255
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Leu Glu Pro Gly Pro Val Thr Ala Gln Val Val Leu Gln Ala Ala Ile
            260                 265                 270

Pro Leu Thr Ser Cys Gly Ser Ser Pro Val Pro Gly Thr Thr Asp Gly
        275                 280                 285

His Arg Pro Thr Ala Glu Ala Pro Asn Thr Thr Ala Gly Gln Val Pro
    290                 295                 300

Thr Thr Glu Val Val Gly Thr Thr Pro Gly Gln Ala Pro Thr Ala Glu
305                 310                 315                 320

Pro Ser Gly Thr Thr Ser Val Gln Val Pro Thr Thr Glu Val Ile Ser
                325                 330                 335

Thr Ala Pro Val Gln Met Pro Thr Ala Glu Ser Thr Gly Met Thr Pro
            340                 345                 350

Glu Lys Val Pro Val Ser Glu Val Met Gly Thr Thr Leu Ala Glu Met
        355                 360                 365

Ser Thr Pro Glu Ala Thr Gly Met Thr Pro Ala Glu Val Ser Ile Val
    370                 375                 380

Val Leu Ser Gly Thr Thr Ala Ala Gln Val Thr Thr Thr Glu Trp Val
385                 390                 395                 400

Glu Thr Thr Ala Arg Glu Leu Pro Ile Pro Glu Pro Glu Gly Pro Asp
                405                 410                 415

Ala Ser Ser Ile Met Ser Thr Glu Ser Ile Thr Gly Ser Leu Gly Pro
            420                 425                 430

Leu Leu Asp Gly Thr Ala Thr Leu Arg Leu Val Lys Arg Gln Val Pro
        435                 440                 445

Leu Asp Cys Val Leu Tyr Arg Tyr Gly Ser Phe Ser Val Thr Leu Asp
    450                 455                 460

Ile Val Gln Gly Ile Glu Ser Ala Glu Ile Leu Gln Ala Val Pro Ser
465                 470                 475                 480

Gly Glu Gly Asp Ala Phe Glu Leu Thr Val Ser Cys Gln Gly Gly Leu
                485                 490                 495

Pro Lys Glu Ala Cys Met Glu Ile Ser Ser Pro Gly Cys Gln Pro Pro
            500                 505                 510

Ala Gln Arg Leu Cys Gln Pro Val Leu Pro Ser Pro Ala Cys Gln Leu
        515                 520                 525

Val Leu His Gln Ile Leu Lys Gly Gly Ser Gly Thr Tyr Cys Leu Asn
    530                 535                 540

Val Ser Leu Ala Asp Thr Asn Ser Leu Ala Val Val Ser Thr Gln Leu
545                 550                 555                 560

Ile Met Pro Gly Gln Glu Ala Gly Leu Gly Gln Val Pro Leu Ile Val
                565                 570                 575

Gly Ile Leu Leu Val Leu Met Ala Val Val Leu Ala Ser Leu Ile Tyr
            580                 585                 590

Arg Arg Arg Leu Met Lys Gln Asp Phe Ser Val Pro Gln Leu Pro His
        595                 600                 605

Ser Ser Ser His Trp Leu Arg Leu Pro Arg Ile Phe Cys Ser Cys Pro
    610                 615                 620

Ile Gly Glu Asn Ser Pro Leu Leu Ser Gly Gln Gln Val
625                 630                 635


<210> SEQ ID NO 28
<211> LENGTH: 637
<212> TYPE: PRT
<213> ORGANISM: Macaca fascicularis

<400> SEQUENCE: 28
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```
Lys Gly Pro Arg Asn Gln Asp Trp Leu Gly Val Ser Arg Gln Leu Arg
1               5                   10                  15

Thr Lys Ala Trp Asn Arg Gln Leu Tyr Pro Glu Trp Thr Glu Ala Gln
            20                  25                  30

Arg Leu Asp Cys Trp Arg Gly Gly Gln Val Ser Leu Lys Val Ser Asn
        35                  40                  45

Asp Gly Pro Thr Leu Ile Gly Ala Asn Ala Ser Phe Ser Ile Ala Leu
    50                  55                  60

Asn Phe Pro Gly Ser Gln Lys Val Leu Pro Asp Gly Gln Val Ile Trp
65                  70                  75                  80

Val Asn Asn Thr Ile Ile Asn Gly Ser Gln Val Trp Gly Gly Gln Pro
                85                  90                  95

Val Tyr Pro Gln Glu Thr Asp Asp Ala Cys Ile Phe Pro Asp Gly Gly
            100                 105                 110

Pro Cys Pro Ser Gly Pro Trp Ser Gln Lys Arg Ser Phe Val Tyr Val
        115                 120                 125

Trp Lys Thr Trp Gly Gln Tyr Trp Gln Val Leu Gly Gly Pro Val Ser
    130                 135                 140

Gly Leu Ser Ile Gly Thr Gly Arg Ala Met Leu Gly Thr His Thr Met
145                 150                 155                 160

Glu Val Thr Val Tyr His Arg Arg Gly Ser Arg Ser Tyr Val Pro Leu
                165                 170                 175

Ala His Ser Ser Ser Ala Phe Thr Ile Thr Asp Gln Val Pro Phe Ser
            180                 185                 190

Val Ser Val Ser Gln Leu Arg Ala Leu Asp Gly Gly Asn Lys His Phe
        195                 200                 205

Leu Arg Asn Gln Pro Leu Thr Phe Ala Leu Gln Leu His Asp Pro Ser
    210                 215                 220

Gly Tyr Leu Ala Glu Ala Asp Leu Ser Tyr Thr Trp Asp Phe Gly Asp
225                 230                 235                 240

Ser Ser Gly Thr Leu Ile Ser Arg Ala Leu Val Val Thr His Thr Tyr
                245                 250                 255

Leu Glu Pro Gly Pro Val Thr Ala Gln Val Val Leu Gln Ala Ala Ile
            260                 265                 270

Pro Leu Thr Ser Cys Gly Ser Ser Pro Val Pro Gly Thr Thr Asp Gly
        275                 280                 285

His Arg Pro Thr Ala Glu Ala Pro Asp Thr Thr Ala Gly Arg Gly Pro
    290                 295                 300

Thr Thr Glu Val Val Gly Thr Thr Pro Gly Gln Val Pro Thr Thr Gln
305                 310                 315                 320

Pro Ser Gly Thr Thr Ser Val Gln Val Pro Thr Thr Glu Val Ile Ser
                325                 330                 335

Thr Thr Pro Val Gln Met Pro Thr Ala Glu Ser Thr Gly Thr Thr Pro
            340                 345                 350

Glu Lys Val Pro Val Ser Glu Val Met Gly Thr Thr Leu Ala Glu Met
        355                 360                 365

Ser Thr Pro Glu Ala Ile Gly Met Thr Pro Ala Glu Val Ser Ile Val
    370                 375                 380

Val Pro Ser Gly Thr Thr Ala Ala Gln Val Thr Thr Thr Glu Trp Val
385                 390                 395                 400

Glu Thr Thr Ala Gly Glu Leu Pro Thr Pro Glu Pro Glu Gly Pro Asp
                405                 410                 415
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```
Thr Ser Ser Ile Met Ser Thr Glu Ser Ile Thr Gly Ser Leu Gly Pro
            420                 425                 430

Leu Leu Asp Gly Thr Ala Thr Leu Arg Leu Glu Lys Arg Gln Val Pro
        435                 440                 445

Leu Asp Cys Val Leu Tyr Arg Tyr Gly Ser Phe Ser Val Thr Leu Asp
    450                 455                 460

Ile Val Gln Gly Ile Glu Ser Ala Glu Ile Leu Gln Val Val Pro Ser
465                 470                 475                 480

Ser Glu Gly Asp Ala Phe Glu Leu Thr Val Ser Cys Gln Gly Gly Leu
                485                 490                 495

Pro Thr Glu Ala Cys Met Glu Ile Ser Ser Pro Gly Cys Gln Pro Pro
            500                 505                 510

Ala Gln Gln Leu Cys Gln Pro Val Pro Pro Ser Pro Ala Cys Gln Leu
        515                 520                 525

Val Leu Tyr Gln Ile Leu Lys Gly Gly Leu Gly Thr Tyr Cys Leu Asn
    530                 535                 540

Val Ser Leu Ala Asp Ala Asn Ser Leu Ala Val Val Ser Thr Gln Leu
545                 550                 555                 560

Ile Val Pro Gly Gln Glu Ala Gly Leu Gly Gln Ala Pro Leu Phe Val
                565                 570                 575

Gly Ile Leu Leu Val Leu Met Ala Val Val Leu Ala Ser Leu Ile Tyr
            580                 585                 590

Arg Arg Arg Leu Met Lys Gln Ala Phe Ser Ile Pro Gln Leu Pro His
        595                 600                 605

Gly Ser Ser His Trp Leu Arg Leu Pro Arg Ile Phe Arg Ser Cys Pro
    610                 615                 620

Ile Gly Glu Asn Ser Pro Leu Leu Ser Gly Gln Glu Val
625                 630                 635


<210> SEQ ID NO 29
<211> LENGTH: 418
<212> TYPE: PRT
<213> ORGANISM: Rattus rattus

<400> SEQUENCE: 29

Met Gly Val Gln Arg Arg Cys Phe Leu Pro Val Leu Val Leu Gly Ala
1               5                   10                  15

Leu Leu Ala Leu Gly Ser Ile Glu Gly Ser Arg Asn Gln Asn Trp His
            20                  25                  30

Gly Val Ser Arg Gln Leu Val Thr Lys Val Trp Asn Lys Gln Leu Tyr
        35                  40                  45

Pro Glu Trp Thr Glu Val Gln Gly Ser Asn Cys Trp Arg Gly Gly Gln
    50                  55                  60

Val Ser Leu Lys Val Arg Asn Asp Gly Pro Thr Leu Val Gly Ala Asn
65                  70                  75                  80

Thr Ser Phe Ser Ile Ala Leu His Phe Pro Gly Ser Gln Lys Val Leu
                85                  90                  95

Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly Ser
            100                 105                 110

Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Arg Glu Pro Asp Asp Ala
        115                 120                 125

Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Pro Lys Pro Pro
    130                 135                 140

Arg Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp Gln
145                 150                 155                 160
```

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```
Val Leu Gly Gly Pro Glu Ser Lys Leu Ser Ile Pro Thr Gly His Ala
                165                 170                 175

Arg Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg Gly
            180                 185                 190

Ser Gln Ser Tyr Val Pro Leu Ala His Ser Ser Ser Thr Phe Thr Ile
        195                 200                 205

Thr Gly Ser Val Ser Arg Leu Leu Asp Asp Thr Asp Thr Ile Met Leu
    210                 215                 220

Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr Gly Ser
225                 230                 235                 240

Phe Ser Leu Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala Glu Ile
                245                 250                 255

Leu Gln Ala Val Pro Ser Ser Glu Gly Asp Ala Phe Glu Leu Thr Val
            260                 265                 270

Ser Cys Arg Gly Gly Leu Pro Lys Glu Ala Cys Met Asp Ile Ser Ser
        275                 280                 285

Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val Pro Pro
    290                 295                 300

Ser Pro Asp Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly Gly Leu
305                 310                 315                 320

Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Ala Asn Ser Leu Ala
                325                 330                 335

Val Ala Ser Thr Gln Leu Val Val Pro Gly Gln Glu Gly Ser Leu Gly
            340                 345                 350

Gln Ala Pro Leu Leu Val Gly Val Leu Leu Val Leu Val Ala Val Val
        355                 360                 365

Leu Ala Ser Leu Ile Tyr Arg His Arg Leu Lys Lys Gln Asp Ser Val
    370                 375                 380

Ser Gln Thr Pro His Gly Ser Thr His Trp Leu Arg Leu Pro Pro Val
385                 390                 395                 400

Phe Cys Ala Arg Arg Leu Gly Glu Ser Ser Pro Leu Leu Ser Gly Gln
                405                 410                 415

Gln Val


<210> SEQ ID NO 30
<211> LENGTH: 393
<212> TYPE: PRT
<213> ORGANISM: Rattus rattus

<400> SEQUENCE: 30

Ser Arg Asn Gln Asn Trp His Gly Val Ser Arg Gln Leu Val Thr Lys
1               5                   10                  15

Val Trp Asn Lys Gln Leu Tyr Pro Glu Trp Thr Glu Val Gln Gly Ser
            20                  25                  30

Asn Cys Trp Arg Gly Gly Gln Val Ser Leu Lys Val Arg Asn Asp Gly
        35                  40                  45

Pro Thr Leu Val Gly Ala Asn Thr Ser Phe Ser Ile Ala Leu His Phe
    50                  55                  60

Pro Gly Ser Gln Lys Val Leu Pro Asp Gly Gln Val Ile Trp Val Asn
65                  70                  75                  80

Asn Thr Ile Ile Asn Gly Ser Gln Val Trp Gly Gly Gln Pro Val Tyr
                85                  90                  95

Pro Arg Glu Pro Asp Asp Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys
            100                 105                 110
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```
Pro Ser Gly Pro Lys Pro Pro Arg Arg Ser Phe Val Tyr Val Trp Lys
        115                 120                 125

Thr Trp Gly Gln Tyr Trp Gln Val Leu Gly Gly Pro Glu Ser Lys Leu
    130                 135                 140

Ser Ile Pro Thr Gly His Ala Arg Leu Gly Thr His Thr Met Glu Val
145                 150                 155                 160

Thr Val Tyr His Arg Arg Gly Ser Gln Ser Tyr Val Pro Leu Ala His
                165                 170                 175

Ser Ser Ser Thr Phe Thr Ile Thr Gly Ser Val Ser Arg Leu Leu Asp
            180                 185                 190

Asp Thr Asp Thr Ile Met Leu Val Lys Arg Gln Val Pro Leu Asp Cys
        195                 200                 205

Val Leu Tyr Arg Tyr Gly Ser Phe Ser Leu Thr Leu Asp Ile Val Gln
    210                 215                 220

Gly Ile Glu Ser Ala Glu Ile Leu Gln Ala Val Pro Ser Ser Glu Gly
225                 230                 235                 240

Asp Ala Phe Glu Leu Thr Val Ser Cys Arg Gly Gly Leu Pro Lys Glu
                245                 250                 255

Ala Cys Met Asp Ile Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg
            260                 265                 270

Leu Cys Gln Pro Val Pro Pro Ser Pro Asp Cys Gln Leu Val Leu His
        275                 280                 285

Gln Ile Leu Lys Gly Gly Leu Gly Thr Tyr Cys Leu Asn Val Ser Leu
    290                 295                 300

Ala Asp Ala Asn Ser Leu Ala Val Ala Ser Thr Gln Leu Val Val Pro
305                 310                 315                 320

Gly Gln Glu Gly Ser Leu Gly Gln Ala Pro Leu Leu Val Gly Val Leu
                325                 330                 335

Leu Val Leu Val Ala Val Val Leu Ala Ser Leu Ile Tyr Arg His Arg
            340                 345                 350

Leu Lys Lys Gln Asp Ser Val Ser Gln Thr Pro His Gly Ser Thr His
        355                 360                 365

Trp Leu Arg Leu Pro Pro Val Phe Cys Ala Arg Arg Leu Gly Glu Ser
    370                 375                 380

Ser Pro Leu Leu Ser Gly Gln Gln Val
385                 390


<210> SEQ ID NO 31
<211> LENGTH: 626
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 31

Met Gly Val Gln Arg Arg Ser Phe Leu Pro Val Leu Val Leu Ser Ala
1               5                   10                  15

Leu Leu Ala Val Gly Ala Leu Glu Gly Ser Arg Asn Gln Asp Trp Leu
            20                  25                  30

Gly Val Pro Arg Gln Leu Val Thr Lys Thr Trp Asn Arg Gln Leu Tyr
        35                  40                  45

Pro Glu Trp Thr Glu Val Gln Gly Ser Asn Cys Trp Arg Gly Gly Gln
    50                  55                  60

Val Ser Leu Arg Val Ile Asn Asp Gly Pro Thr Leu Val Gly Ala Asn
65                  70                  75                  80

Ala Ser Phe Ser Ile Ala Leu His Phe Pro Gly Ser Gln Lys Val Leu
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                85                  90                  95

Pro Asp Gly Gln Val Ile Trp Ala Asn Asn Thr Ile Ile Asn Gly Ser
            100                 105                 110

Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Pro Asp Asp Ala
        115                 120                 125

Cys Val Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Pro Lys Pro Pro
    130                 135                 140

Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Lys Tyr Trp Gln
145                 150                 155                 160

Val Leu Gly Gly Pro Val Ser Arg Leu Ser Ile Ala Thr Gly His Ala
                165                 170                 175

Lys Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg Gly
            180                 185                 190

Ser Gln Ser Tyr Val Pro Leu Ala His Ala Ser Ser Thr Phe Thr Ile
        195                 200                 205

Thr Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Gln Ala Leu
    210                 215                 220

Asp Gly Glu Thr Lys His Phe Leu Arg Asn His Pro Leu Ile Phe Ala
225                 230                 235                 240

Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu Ser
                245                 250                 255

Tyr Thr Trp Asp Phe Gly Asp Gly Thr Gly Thr Leu Ile Ser Arg Ala
            260                 265                 270

Leu Asp Val Thr His Thr Tyr Leu Glu Ser Gly Ser Val Thr Ala Gln
        275                 280                 285

Val Val Leu Gln Ala Ala Ile Pro Leu Val Ser Cys Gly Ser Ser Pro
    290                 295                 300

Val Pro Gly Thr Thr Asp Gly Tyr Met Pro Thr Ala Glu Ala Pro Gly
305                 310                 315                 320

Thr Thr Ser Arg Gln Gly Thr Thr Thr Lys Val Val Gly Thr Thr Pro
                325                 330                 335

Gly Gln Met Pro Thr Thr Gln Pro Ser Gly Thr Thr Val Val Gln Met
            340                 345                 350

Pro Thr Thr Glu Val Thr Ala Thr Thr Ser Glu Gln Met Leu Thr Ser
        355                 360                 365

Ala Val Ile Asp Thr Thr Leu Ala Glu Val Ser Thr Thr Glu Gly Thr
    370                 375                 380

Gly Thr Thr Pro Thr Arg Pro Ser Gly Thr Thr Val Ala Gln Ala Thr
385                 390                 395                 400

Thr Thr Glu Gly Pro Asp Ala Ser Pro Leu Leu Pro Thr Gln Ser Ser
                405                 410                 415

Thr Gly Ser Ile Ser Pro Leu Leu Asp Asp Thr Asp Thr Ile Met Leu
            420                 425                 430

Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr Gly Ser
        435                 440                 445

Phe Ser Leu Ala Leu Asp Ile Val Gln Gly Ile Glu Ser Ala Glu Ile
    450                 455                 460

Leu Gln Ala Val Pro Phe Ser Glu Gly Asp Ala Phe Glu Leu Thr Val
465                 470                 475                 480

Ser Cys Gln Gly Gly Leu Pro Lys Glu Ala Cys Met Asp Ile Ser Ser
                485                 490                 495

Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Ser Val Pro Pro
            500                 505                 510
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Ser Pro Asp Cys Gln Leu Val Leu His Gln Val Leu Lys Gly Gly Ser
        515                 520                 525

Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Ala Asn Ser Leu Ala
    530                 535                 540

Val Ala Ser Thr Gln Leu Val Val Pro Gly Gln Asp Gly Gly Leu Gly
545                 550                 555                 560

Gln Ala Pro Leu Leu Val Gly Ile Leu Leu Val Leu Val Ala Val Val
                565                 570                 575

Leu Ala Ser Leu Ile His Arg His Arg Leu Lys Lys Gln Gly Ser Val
            580                 585                 590

Ser Gln Met Pro His Gly Ser Thr His Trp Leu Arg Leu Pro Pro Val
        595                 600                 605

Phe Arg Ala Arg Gly Leu Gly Glu Asn Ser Pro Leu Leu Ser Gly Gln
    610                 615                 620

Gln Val
625


<210> SEQ ID NO 32
<211> LENGTH: 601
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 32

Ser Arg Asn Gln Asp Trp Leu Gly Val Pro Arg Gln Leu Val Thr Lys
1               5                   10                  15

Thr Trp Asn Arg Gln Leu Tyr Pro Glu Trp Thr Glu Val Gln Gly Ser
            20                  25                  30

Asn Cys Trp Arg Gly Gly Gln Val Ser Leu Arg Val Ile Asn Asp Gly
        35                  40                  45

Pro Thr Leu Val Gly Ala Asn Ala Ser Phe Ser Ile Ala Leu His Phe
    50                  55                  60

Pro Gly Ser Gln Lys Val Leu Pro Asp Gly Gln Val Ile Trp Ala Asn
65                  70                  75                  80

Asn Thr Ile Ile Asn Gly Ser Gln Val Trp Gly Gly Gln Pro Val Tyr
                85                  90                  95

Pro Gln Glu Pro Asp Asp Ala Cys Val Phe Pro Asp Gly Gly Pro Cys
            100                 105                 110

Pro Ser Gly Pro Lys Pro Pro Lys Arg Ser Phe Val Tyr Val Trp Lys
        115                 120                 125

Thr Trp Gly Lys Tyr Trp Gln Val Leu Gly Gly Pro Val Ser Arg Leu
    130                 135                 140

Ser Ile Ala Thr Gly His Ala Lys Leu Gly Thr His Thr Met Glu Val
145                 150                 155                 160

Thr Val Tyr His Arg Arg Gly Ser Gln Ser Tyr Val Pro Leu Ala His
                165                 170                 175

Ala Ser Ser Thr Phe Thr Ile Thr Asp Gln Val Pro Phe Ser Val Ser
            180                 185                 190

Val Ser Gln Leu Gln Ala Leu Asp Gly Glu Thr Lys His Phe Leu Arg
        195                 200                 205

Asn His Pro Leu Ile Phe Ala Leu Gln Leu His Asp Pro Ser Gly Tyr
    210                 215                 220

Leu Ala Glu Ala Asp Leu Ser Tyr Thr Trp Asp Phe Gly Asp Gly Thr
225                 230                 235                 240

Gly Thr Leu Ile Ser Arg Ala Leu Asp Val Thr His Thr Tyr Leu Glu
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               245                 250                 255

Ser Gly Ser Val Thr Ala Gln Val Val Leu Gln Ala Ala Ile Pro Leu
            260                 265                 270

Val Ser Cys Gly Ser Ser Pro Val Pro Gly Thr Thr Asp Gly Tyr Met
        275                 280                 285

Pro Thr Ala Glu Ala Pro Gly Thr Thr Ser Arg Gln Gly Thr Thr Thr
    290                 295                 300

Lys Val Val Gly Thr Thr Pro Gly Gln Met Pro Thr Thr Gln Pro Ser
305                 310                 315                 320

Gly Thr Thr Val Val Gln Met Pro Thr Thr Glu Val Thr Ala Thr Thr
                325                 330                 335

Ser Glu Gln Met Leu Thr Ser Ala Val Ile Asp Thr Thr Leu Ala Glu
            340                 345                 350

Val Ser Thr Thr Glu Gly Thr Gly Thr Thr Pro Thr Arg Pro Ser Gly
        355                 360                 365

Thr Thr Val Ala Gln Ala Thr Thr Thr Glu Gly Pro Asp Ala Ser Pro
    370                 375                 380

Leu Leu Pro Thr Gln Ser Ser Thr Gly Ser Ile Ser Pro Leu Leu Asp
385                 390                 395                 400

Asp Thr Asp Thr Ile Met Leu Val Lys Arg Gln Val Pro Leu Asp Cys
                405                 410                 415

Val Leu Tyr Arg Tyr Gly Ser Phe Ser Leu Ala Leu Asp Ile Val Gln
            420                 425                 430

Gly Ile Glu Ser Ala Glu Ile Leu Gln Ala Val Pro Phe Ser Glu Gly
        435                 440                 445

Asp Ala Phe Glu Leu Thr Val Ser Cys Gln Gly Gly Leu Pro Lys Glu
    450                 455                 460

Ala Cys Met Asp Ile Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg
465                 470                 475                 480

Leu Cys Gln Ser Val Pro Pro Ser Pro Asp Cys Gln Leu Val Leu His
                485                 490                 495

Gln Val Leu Lys Gly Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu
            500                 505                 510

Ala Asp Ala Asn Ser Leu Ala Val Ala Ser Thr Gln Leu Val Val Pro
        515                 520                 525

Gly Gln Asp Gly Gly Leu Gly Gln Ala Pro Leu Leu Val Gly Ile Leu
    530                 535                 540

Leu Val Leu Val Ala Val Val Leu Ala Ser Leu Ile His Arg His Arg
545                 550                 555                 560

Leu Lys Lys Gln Gly Ser Val Ser Gln Met Pro His Gly Ser Thr His
                565                 570                 575

Trp Leu Arg Leu Pro Pro Val Phe Arg Ala Arg Gly Leu Gly Glu Asn
            580                 585                 590

Ser Pro Leu Leu Ser Gly Gln Gln Val
        595                 600


<210> SEQ ID NO 33
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 33

Gly Tyr Thr Phe Thr Ser Tyr Trp Met Gln
1               5                   10
```

<210> SEQ ID NO 34
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 34

Thr Ile Tyr Pro Gly Asp Gly Asp Thr Ser Tyr Ala Gln Lys Phe Lys
1 5 10 15

Gly

<210> SEQ ID NO 35
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 35

Trp Gly Tyr Ala Tyr Asp Ile Asp Asn
1 5

<210> SEQ ID NO 36
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 36

Lys Ser Ser Gln Ser Leu Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn
1 5 10 15

<210> SEQ ID NO 37
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 37

Leu Val Ser Lys Leu Asp Ser
1 5

<210> SEQ ID NO 38
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 38

Trp Gln Gly Thr His Phe Pro Tyr Thr
1 5

<210> SEQ ID NO 39
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 39

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Lys Pro Gly Lys Ala
35 40 45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60

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Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65                  70                  75                  80

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Trp Gln Gly
                85                  90                  95

Thr His Phe Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110


<210> SEQ ID NO 40
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 40

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Trp Met Gln Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Thr Ile Tyr Pro Gly Asp Gly Asp Thr Ser Tyr Ala Gln Lys Phe
    50                  55                  60

Lys Gly Arg Ala Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Trp Gly Tyr Ala Tyr Asp Ile Asp Asn Trp Gly
            100                 105


<210> SEQ ID NO 41
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 41

Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val Thr Ile Gly
1               5                   10                  15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
            20                  25                  30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Thr Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Trp Gln Gly
                85                  90                  95

Thr His Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


<210> SEQ ID NO 42
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 42

Gln Val Gln Leu Leu Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1               5                   10                  15
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Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Trp Met Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Thr Ile Tyr Pro Gly Asp Gly Asp Thr Ser Tyr Ala Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Thr Asp Lys Tyr Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Trp Gly Tyr Ala Tyr Asp Ile Asp Asn Trp Gly
            100                 105


<210> SEQ ID NO 43
<211> LENGTH: 219
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 43

Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val Thr Ile Gly
1               5                   10                  15

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
            20                  25                  30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Thr Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Trp Gln Gly
                85                  90                  95

Thr His Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210> SEQ ID NO 44
<211> LENGTH: 448
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
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<400> SEQUENCE: 44

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Gln Val Gln Leu Leu Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1               5                  10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Trp Met Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Thr Ile Tyr Pro Gly Asp Gly Asp Thr Ser Tyr Ala Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Thr Asp Lys Tyr Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Trp Gly Tyr Ala Tyr Asp Ile Asp Asn Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
    210                 215                 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            260                 265                 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            340                 345                 350

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360                 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                405                 410                 415
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Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420                 425                 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445


<210> SEQ ID NO 45
<211> LENGTH: 219
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 45

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
            20                  25                  30

Asp Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Lys Pro Gly Lys Ala
        35                  40                  45

Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
    50                  55                  60

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65                  70                  75                  80

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Trp Gln Gly
                85                  90                  95

Thr His Phe Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210> SEQ ID NO 46
<211> LENGTH: 448
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 46

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Trp Met Gln Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Thr Ile Tyr Pro Gly Asp Gly Asp Thr Ser Tyr Ala Gln Lys Phe
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    50                  55                  60

Lys Gly Arg Ala Thr Leu Ser Thr Asp Lys Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Trp Gly Tyr Ala Tyr Asp Ile Asp Asn Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
    210                 215                 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            260                 265                 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            340                 345                 350

Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360                 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                405                 410                 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420                 425                 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445


<210> SEQ ID NO 47
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 47

Asp Val Gln Ile Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser Pro Gly
1 5 10 15

Glu Thr Ile Thr Ile Asn Cys Arg Ala Thr Lys Ser Ile Ser Lys Tyr
20 25 30

Leu Ala Trp Tyr Gln Glu Gln Pro Gly Lys Thr Asn Asn Leu Leu Ile
35 40 45

Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Tyr Pro Tyr
85 90 95

Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> SEQ ID NO 48
<211> LENGTH: 449
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 48

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Met Lys Ile Ser Cys Lys Ser Ser Gly Tyr Ser Phe Thr Arg Tyr
20 25 30

Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp Ile
35 40 45

Gly Val Ile Asn Pro Tyr Asn Gly Gly Thr Val Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Thr Asp Tyr Asp Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Ser Val Thr Val Ser Ser Ala Lys Thr Lys Gly Pro Ser Val Phe

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        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys


<210> SEQ ID NO 49
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 49

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Ser Met Arg Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
            20                  25                  30

Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp Ile
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-continued

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        35                  40                  45

Gly Val Tyr Asn Pro Tyr Asn Gly Gly Thr Val Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Thr Tyr
65                  70                  75                  80

Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
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            100                 105                 110

Thr Ser Val Thr Val Ser Ser
        115


<210> SEQ ID NO 50
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 50

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1               5                   10                  15

Ser Met Lys Ile Ser Cys Lys Ser Ser Gly Tyr Ser Phe Thr Arg Tyr
            20                  25                  30

Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp Ile
        35                  40                  45

Gly Val Ile Asn Pro Tyr Asn Gly Gly Thr Val Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

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            100                 105                 110

Thr Ser Val Thr Val Ser Ser
        115


<210> SEQ ID NO 51
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 51

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1               5                   10                  15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Lys Tyr
            20                  25                  30

Gly Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45

Gly Val Ile Trp Ala Gly Gly Asn Thr Asn Tyr Asn Ser Ala Leu Met
    50                  55                  60

Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65                  70                  75                  80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
                85                  90                  95

Thr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
            100                 105                 110
```

What is claimed is:

1. An isolated monoclonal antibody that binds to PMEL17, wherein the antibody binds an epitope within amino acids 105 to 125 of SEQ ID NO: 26, or binds to an epitope within amino acids 25 to 45 of SEQ ID NO: 26.

2. The antibody of claim 1, which is a human, humanized, or chimeric antibody.

3. The antibody of claim 1, which is an antibody fragment that binds PMEL17.

4. The antibody of claim 1, wherein PMEL17 is human PMEL17.

5. The antibody of claim 4, wherein human PMEL17 has the sequence of SEQ ID NO: 26 or SEQ ID NO: 27.

6. The antibody of claim 1, wherein the antibody comprises:

a) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or

b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or

c) HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 of the antibody produced by hybridoma 7509 (31D1.6.7) having ATCC Accession No. PTA-12862.

7. The antibody of claim 6, wherein the antibody comprises:

a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1 and a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 2; or

b) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9 and a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10; or

c) a VH sequence having at least 95% sequence identity to the VH sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862 and a VL sequence having at least 95% sequence identity to the VL sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

8. An isolated antibody comprising (a) a VH sequence having the amino acid sequence of SEQ ID NO: 1 and a VL sequence having the amino acid sequence of SEQ ID NO: 2; or (b) a VH sequence having the amino acid sequence of SEQ ID NO: 9 and a VL sequence having the amino acid sequence of SEQ ID NO: 10; or (c) a VH sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862 and a VL sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

9. An isolated antibody that binds to PMEL17, wherein the antibody comprises:

a) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 20, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 22, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 23, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 24, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 25; or

b) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11 and a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 12, wherein the VH sequence comprises HVR-H1, HVR-H2, and HVR-H3 of SEQ ID NOs: 20, 21, and 22, respectively, and the VL sequence comprises HVR-L1, HVR-L2, and HVR-L3 of SEQ ID NOs: 23, 24, and 25, respectively; or

c) a VH sequence having the amino acid sequence of SEQ ID NO: 11 and a VL sequence having the amino acid sequence of SEQ ID NO: 1.

10. The antibody of claim 1, which is an IgG1, IgG2a or IgG2b antibody.

11. An immunoconjugate comprising the antibody of claim 1 and a cytotoxic agent.

12. The immunoconjugate of claim 11 having the formula Ab-(L-D)p, wherein:

(a) Ab is the antibody of claim 1;

(b) L is a linker;

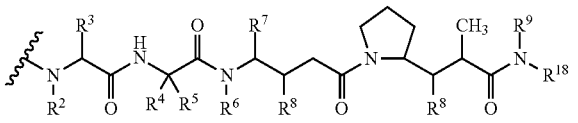
(c) D is a drug selected from a maytansinoid, an auristatin, a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative; and

(d) p ranges from 1-8.

13. The immunoconjugate of claim 12, wherein D is an auristatin.

14. The immunoconjugate of claim 13, wherein D has formula D_E

D_E

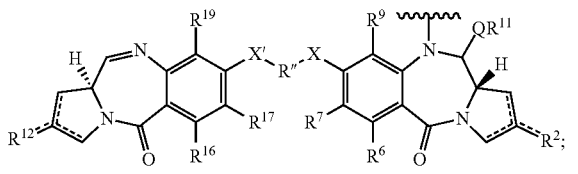


and wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^5 is H, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O—CH_3, OH, and H; R^9 is H; and R^{18} is —$C(R^8)_2$—$C(R^8)_2$-aryl.

15. The immunoconjugate of claim 12, wherein the drug is MMAE.

16. The immunoconjugate of claim 12, wherein D is a pyrrolobenzodiazepine of Formula A:

A



wherein the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R^2 is independently selected from H, OH, =O, =CH_2, CN, R, OR, =CH—R^D, =$C(R^D)_2$, O—SO_2—R, CO_2R and COR, and optionally further selected from halo or dihalo, wherein

R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

Q is independently selected from 0, S and NH;

R^{11} is either H, or R or, where Q is 0, SO_3M, where M is a metal cation;

R and R' are each independently selected from optionally substituted C_{1-8} alkyl, C_{3-8} heterocyclyl and C_{5-20} aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

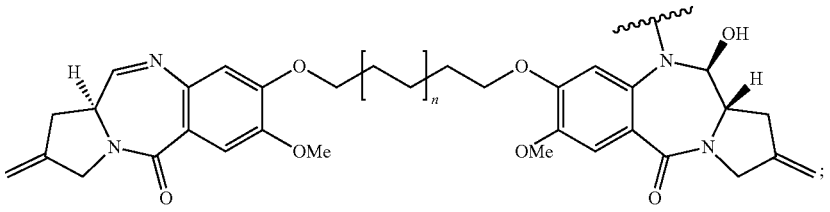
R^{12}, R^{16}, R^{19} and R^{17} are as defined for R^2, R^6, R^9 and R^7 respectively;

R" is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms and/or aromatic rings that are optionally substituted; and

X and X' are independently selected from O, S and N(H).

17. The immunoconjugate of claim **16**, wherein D has the structure:

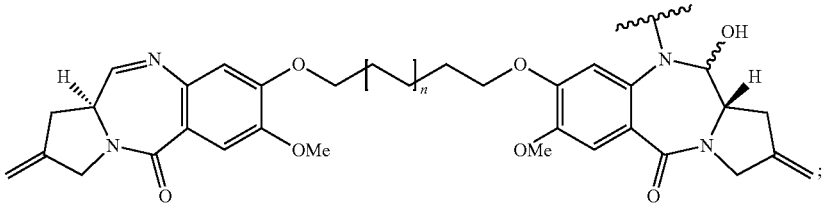
A(II)



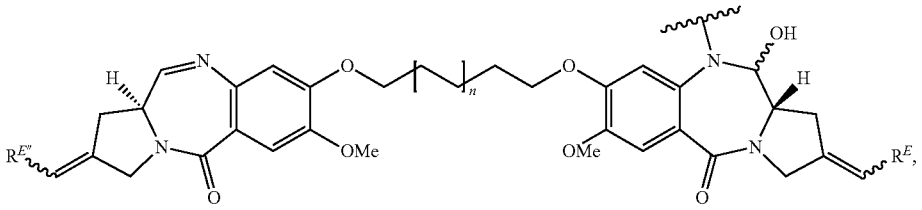
wherein n is 0 or 1.

18. The immunoconjugate of claim **16**, wherein D has a structure selected from:

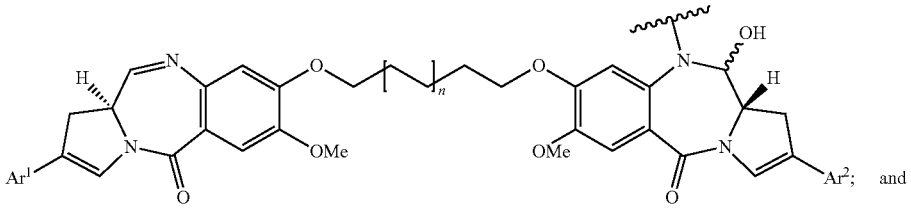
A(I)



A(III)

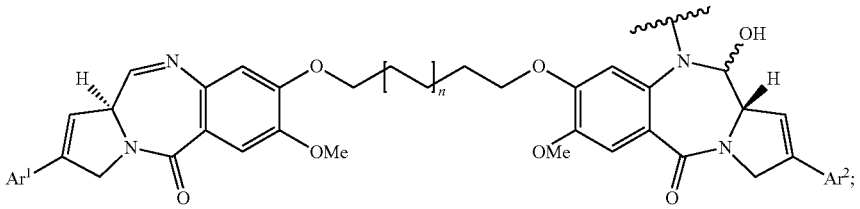


A(IV)



and

A(V)

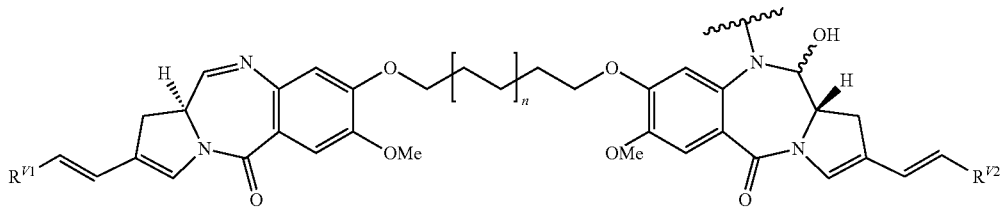


wherein R^E and $R^{E''}$ are each independently selected from H or R^D, wherein R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

wherein Ar^1 and Ar^2 are each independently optionally substituted C_{5-20} aryl; and

wherein n is 0 or 1.

19. The immunoconjugate of claim **12**, wherein D is a pyrrolobenzodiazepine of Formula B:



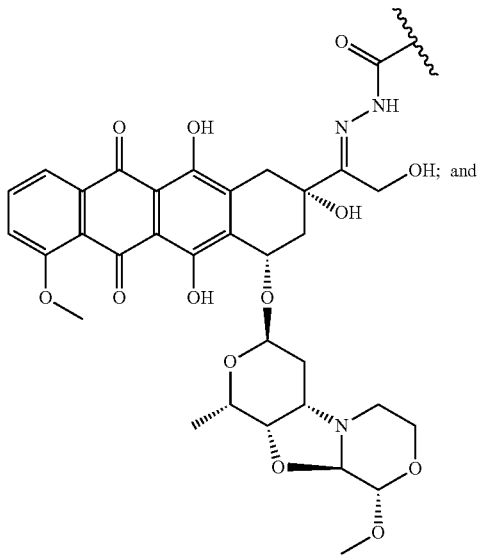
wherein the horizontal wavy line indicates the covalent attachement site to the linker;

R^{V1} and R^{V2} are independently selected from H, methyl, ethyl, phenyl, fluoro-substituted phenyl, and C_{5-6} heterocyclyl; and

n is 0 or 1.

20. The immunoconjugate of claim **12**, wherein D is a nemorubicin derivative.

21. The immunoconjugate of claim **20**, wherein D has a structure selected from:



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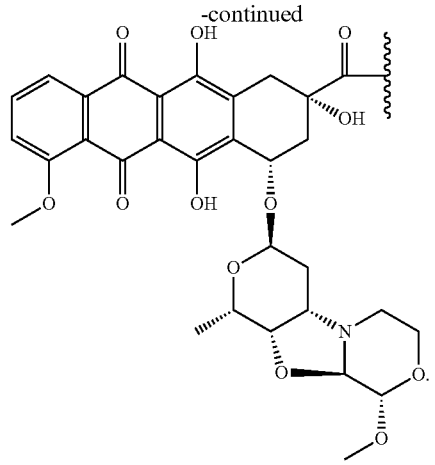


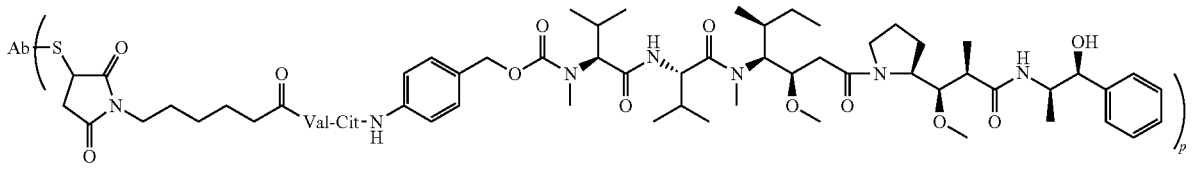
22. The immunoconjugate of claim **12**, wherein the linker is cleavable by a protease.

23. The immunoconjugate of claim **22**, wherein the linker comprises a val-cit dipeptide or a Phe-Lys dipeptide.

24. The immunoconjugate of claim **12**, wherein the linker is acid-labile.

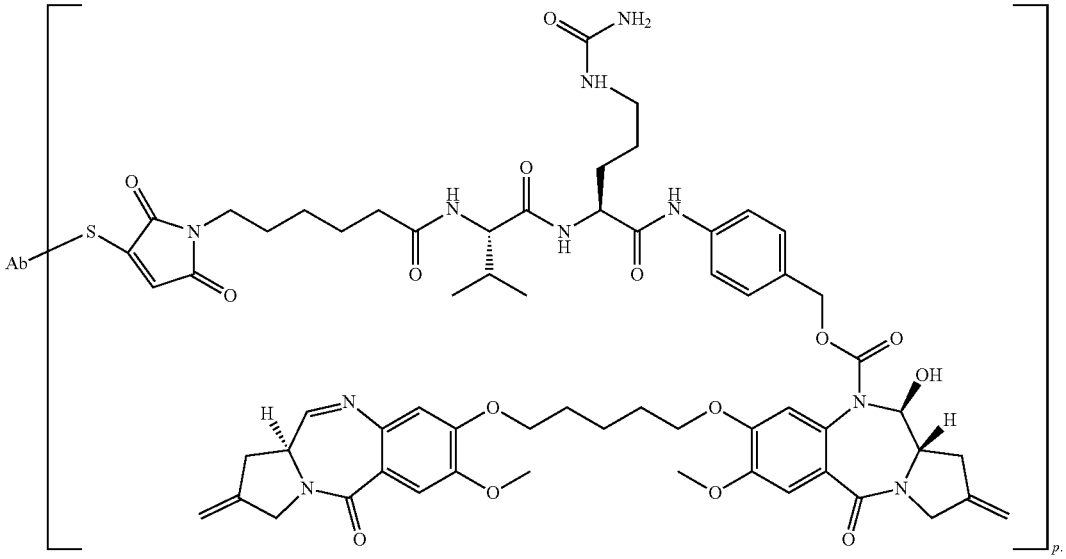
25. The immunoconjugate of claim **24**, wherein the linker comprises hydrazone.

26. The immunoconiugate of claim **14** having the formula:

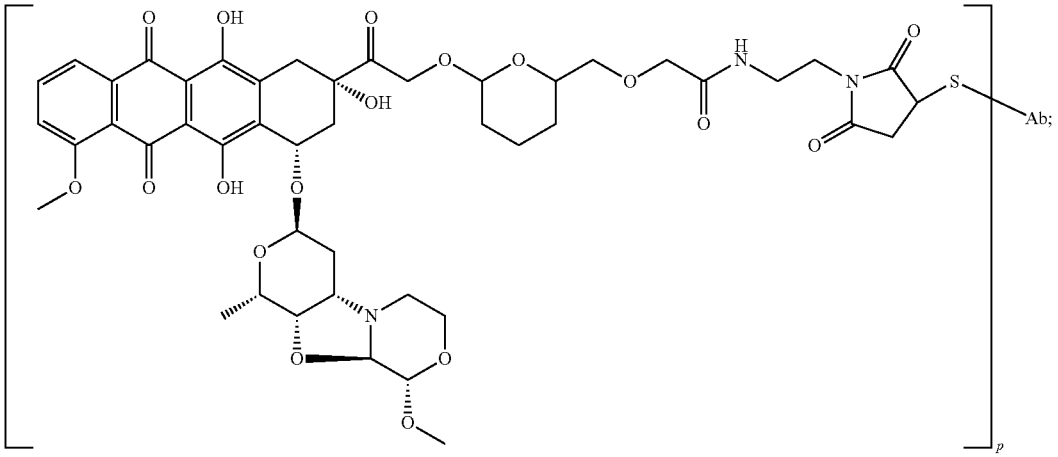


wherein S is a sulfur atom.

27. The immunoconjugate of claim **17** having the formula:



28. The immunoconjugate of claim **21** having a formula selected from:



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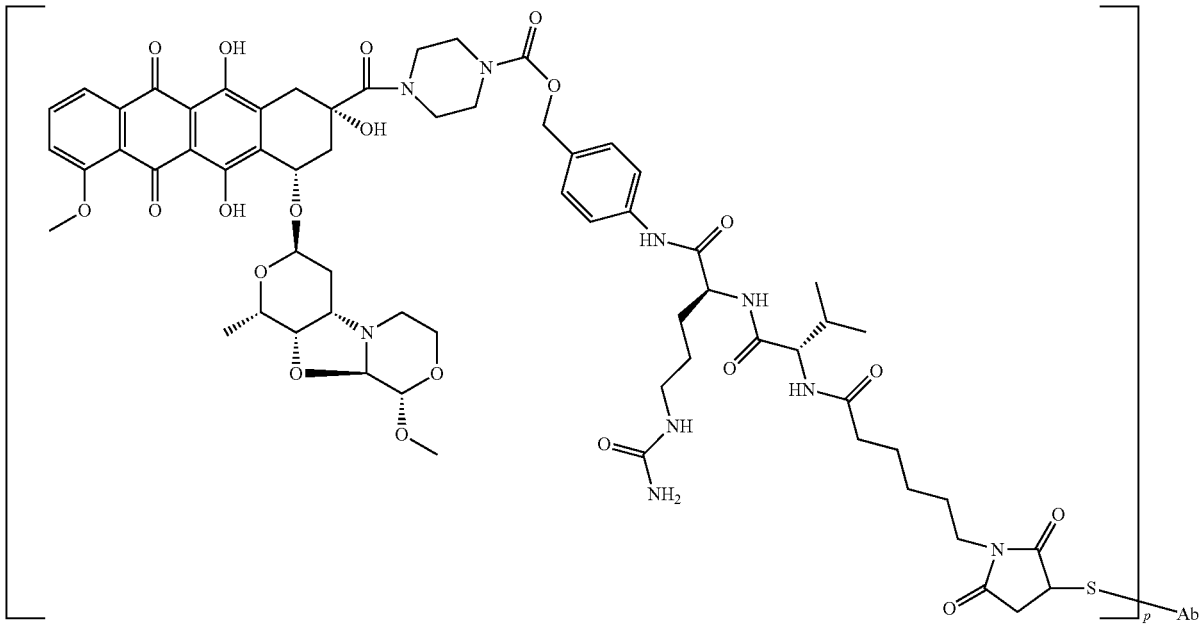

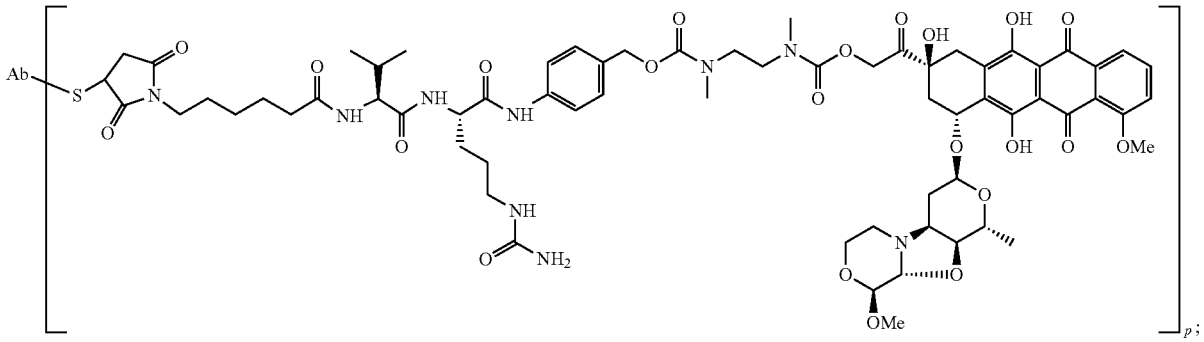

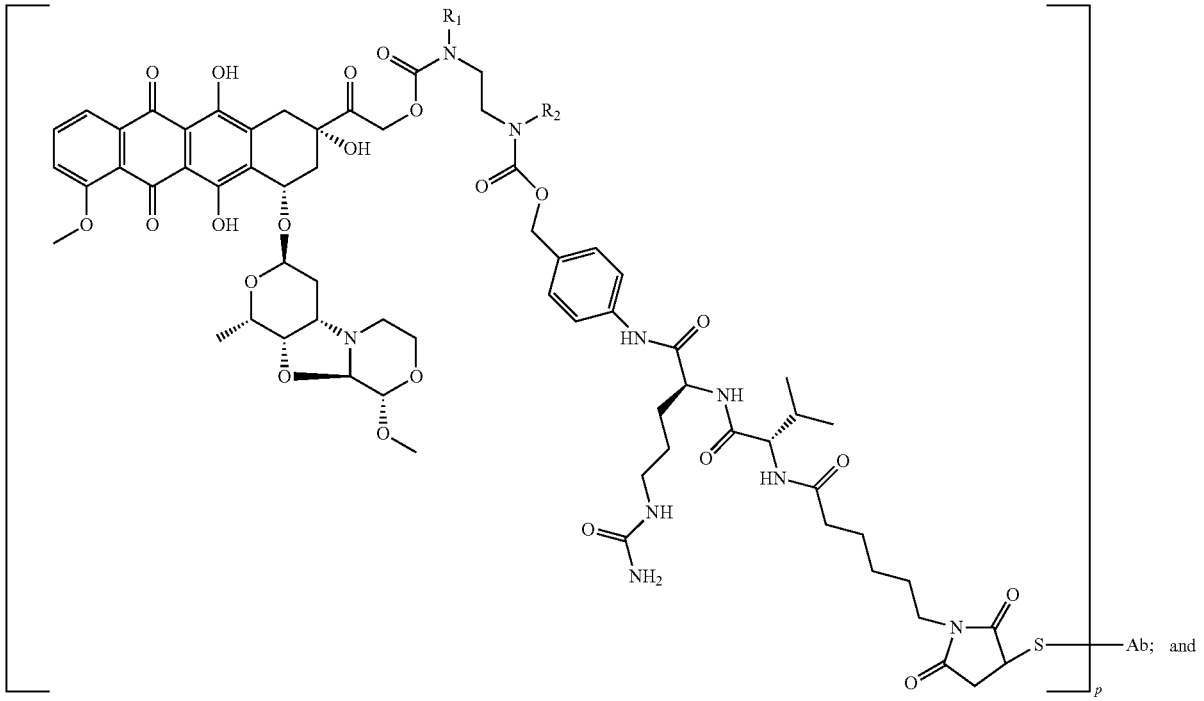

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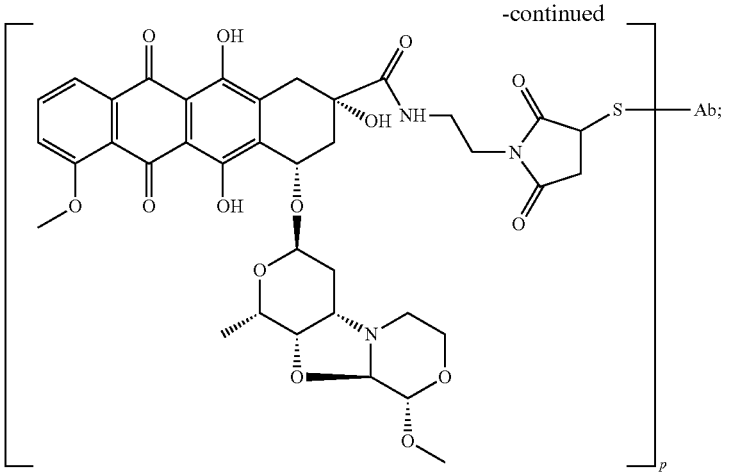

wherein R_1 and R_2 are independently selected from H and C_1-C_6 alkyl.

29. The immunoconjugate of claim **12**, wherein p ranges from 2-5.

30. The immunoconjugate of claim **12**, comprising an antibody that comprises:

a) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 3, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 4, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 5, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 6, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or

b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 7, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 8; or

c) HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2, and HVR-L3 of the antibody produced by hybridoma 7509 (31D1.6.7) having ATCC Accession No. PTA-12862.

31. The immunoconjugate of claim **12**, comprising an antibody that comprises (a) a VH sequence having the amino acid sequence of SEQ ID NO: 1 and a VL sequence having the amino acid sequence of SEQ ID NO: 2; or (b) a VH sequence having the amino acid sequence of SEQ ID NO: 9 and a VL sequence having the amino acid sequence of SEQ ID NO: 10; or (c) a VH sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862 and a VL sequence of the antibody produced by hybridoma 7509(31D1.6.7) having ATCC Accession No. PTA-12862.

32. A pharmaceutical formulation comprising the immunoconjugate of claim **12** and a pharmaceutically acceptable carrier.

33. The pharmaceutical formulation of claim **32**, further comprising an additional therapeutic agent.

34. A method of treating an individual having a PMEL17-positive cancer, the method comprising administering to the individual an effective amount of the immunoconjugate of claim **11**.

35. The method of claim **34**, wherein the PMEL17-positive cancer is melanoma.

36. The method of claim **35**, further comprising administering an additional therapeutic agent to the individual.

37. A method of inhibiting proliferation of a PMEL17-positive cell, the method comprising exposing the cell to the immunoconjugate of claim **11** under conditions permissive for binding of the immunoconjugate to PMEL17 on the surface of the cell, thereby inhibiting proliferation of the cell.

38. The method of claim **37**, wherein the cell is a melanoma cell.

39. The antibody of claim **1** conjugated to a label.

40. The antibody of claim **39**, wherein the label is a positron emitter.

41. The antibody of claim **40**, wherein the positron emitter is ^{89}Zr.

42. A method of detecting human PMEL17 in a biological sample comprising contacting the biological sample with the anti-PMEL17 antibody of claim **1** under conditions permissive for binding of the anti-PMEL17 antibody to a naturally occurring human PMEL17, and detecting whether a complex is formed between the anti-PMEL17 antibody and a naturally occurring human PMEL17 in the biological sample.

43. The method of claim **42**, wherein the biological sample is a melanoma sample.

44. A method for detecting a PMEL17-positive cancer comprising (i) administering a labeled anti-PMEL17 antibody to a subject having or suspected of having a PMEL17-positive cancer, wherein the labeled anti-PMEL17 antibody comprises the anti-PMEL17 antibody of claim **1**, and (ii) detecting the labeled anti-PMEL17 antibody in the subject, wherein detection of the labeled anti-PMEL17 antibody indicates a PMEL17-positive cancer in the subject.

45. The method of claim **44**, wherein the labeled anti-PMEL17 antibody comprises an anti-PMEL17 antibody conjugated to a positron emitter.

46. The method of claim **45**, wherein the positron emitter is ^{89}Zr.

47. A method of treating an individual having a PMEL17-positive cancer, wherein the PMEL17-positive cancer is resistant to a first therapeutic, the method comprising administering to the individual an effective amount of the immunoconjugate of claim **11**.

48. The method of claim **47**, wherein the PMEL17-positive cancer is melanoma.

49. The method of claim **47**, wherein the first therapeutic comprises a first antibody that binds an antigen other than PMEL17.

50. The method of claim 49, wherein the first therapeutic is a first immunoconjugate comprising a first antibody that binds an antigen other than PMEL17 and a first cytotoxic agent.

51. The method of claim 49, wherein the first antibody binds an antigen selected from endothelin B receptor (ETBR), tyrosinase-related protein 1 (TYRP1), cytotoxic T lymphocyte antigen 4 (CTLA-4), and glycoprotein NMB (GPNMB).

52. The method of claim 51, wherein the first antibody binds ETBR.

53. The method of claim 52, wherein the first antibody is hu5E9.v1.

54. The method of claim 53, wherein the first immunoconjugate is hu5E9.v1-MC-val-cit-PAB-MMAE.

55. The method of claim 50, wherein the first cytotoxic agent and the cytotoxic agent of the immunoconjugate comprising an antibody that binds to PMEL17 are different.

56. The method of claim 55, wherein the first cytotoxic agent is MMAE and the cytotoxic agent of the immunoconjugate comprising an antibody that binds to PMEL17 is selected from a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative.

57. The method of claim 56, wherein the cytotoxic agent of the immunoconjugate comprising an antibody that binds to PMEL17 is selected from a pyrrolobenzodiazepine and a nemorubicin derivative.

58. A method of treating an individual with PMEL17-positive cancer, comprising administering to the individual an effective amount of a first immunoconjugate of claim 11 in combination with a second immunoconjugate comprising an antibody that binds ETBR.

59. The method of claim 58, wherein the antibody that binds ETBR comprises an HVR H1 comprising a sequence of SEQ ID NO: 33, an HVR H2 comprising a sequence of SEQ ID NO: 34, an HVR H3 comprising a sequence of SEQ ID NO: 35, an HVR L1 comprising a sequence of SEQ ID NO: 36, an HVR L2 comprising a sequence of SEQ ID NO: 37, and an HVR L3 comprising a sequence of SEQ ID NO: 38.

60. The method of claim 58, wherein the antibody that binds ETBR comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 40 and a light chain variable region comprising the sequence of SEQ ID NO: 39.

61. The method of claim 58, wherein the first immunoconjugate comprises a cytotoxic agent selected from an auristatin, a pyrrolobenzodiazepine, and a nemorubicin derivative, and the second immunoconjugate comprises a cytotoxic agent selected from an auristatin, a pyrrolobenzodiazepine, and a nemorubicin derivative.

62. The method of claim 58, wherein the first immunoconjugate comprises a cytotoxic agent selected from a pyrrolobenzodiazepine and a nemorubicin derivative, and the second immunoconjugate comprises an auristatin.

63. The method of claim 62, wherein the second immunoconjugate comprises MMAE.

64. The method of claim 58, wherein the second immunoconjugate comprises a linker-drug portion comprising MC-val-cit-PAB-MMAE.

65. The method of claim 58, wherein the second immunoconjugate is hu5E9.v1-MC-val-cit-PAB-MMAE.

66. The method of claim 58, wherein the PMEL17-positive cancer is melanoma.

67. The method of claim 58, wherein the PMEL17-positive cancer is also ETBR-positive.

* * * * *